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Review Article

State of the art in bile analysis in forensic toxicology

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ABSTRACT

In forensic toxicology, alternative matrices to blood are useful in case of limited, unavailable or unusable blood sample, suspected postmortem redistribution or long drug intake-to-sampling interval. The present article provides an update on the state of knowledge for the use of bile in forensic toxicology, through a review of the Medline literature from 1970 to May 2015. Bile physiology and technical aspects of analysis (sampling, storage, sample preparation and analytical methods) are reported, to highlight specificities and consequences from an analytical and interpretative point of view. A table summarizes cause of death and quantification in bile and blood of 133 compounds from more than 200 case reports, providing a useful tool for forensic physicians and toxicologists involved in interpreting bile analysis. Qualitative and quantitative interpretation is discussed. As bile/blood concentration ratios are high for numerous molecules or metabolites, bile is a matrix of choice for screening when blood concentrations are low or non-detectable: e.g., cases of weak exposure or long intake-to-death interval. Quantitative applications have been little investigated, but small molecules with low bile/blood concentration ratios seem to be good candidates for quantitative bile-based interpretation. Further experimental data on the mechanism and properties of biliary extraction of xenobiotics of forensic interest are required to improve quantitative interpretation.

Contents

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1. Introduction

Bile is a complex greenish-yellow liquid secreted by the hepatocytes of the liver. Outside of mealtimes, it is stored in the gallbladder, and released into the duodenum during digestion notably to facilitate lipid absorption by the intestine [1] and protect the intestinal mucosa against gastric acid [2]. It is also a medium of excretion of endogenous substances such as bilirubin, a breakdown product of hemoglobin, or excess cholesterol and of exogenous substances such as heavy metals and drugs [3]. Thus, bile contributes, along with metabolism, to liver purification and is an elimination pathway for xenobiotics which, once excreted into the bile, are eliminated via the feces. The biliary system is involved in the first-pass effect of the liver, which consists in the metabolism or excretion of certain xenobiotics absorbed from the digestive system before they can reach the systemic blood circulation. Enterohepatic circulation is possible for some endogenous (e.g. bile salts) or exogenous molecules (morphine, colchicine, etc.) that are excreted into the bile but then reabsorbed in the intestine, to enter the general circulation or else be excreted again [4–6].

Bile has been studied as a postmortem matrix for screening for human chorionic gonadotropin (βhCG) [7], human immunodeficiency virus (HIV) [8], insulin [9–11] or blood group [12]. Biliary excretion of metals and its role in their potential toxicity have also been a focus of studies [1,13–15]. Bile is especially an additional autopsy sample for toxicologic analysis alongside other matrices (central blood, peripheral blood, stomach contents, etc.) or in the absence of analyzable blood. It is easy to sample postmortem from the gallbladder and tends to show high levels of xenobiotics, so that it is often seen as an interesting matrix for wide-scale screening [16,17].

Understanding, assessing and possibly anticipating a molecule's behavior in terms of biliary elimination is very useful for the development of new treatments as well as in forensics to help interpret bile results. Biliary excretion, however, is complex and difficult to study. In vivo analysis obviously runs up against problems of access [3,18]. Studies are therefore often performed in animal models or in vitro (perfused liver, hepatocyte culture, hepatocyte membrane reconstitution), entailing issues of extrapolation. In vivo sampling is, nevertheless, feasible via T-tubes fitted in cholecystectomy patients [19], but such studies are biased by the presence of hepatobiliary pathology. Postmortem data involve the problem of wide variability in forensic populations and the lack of information regarding xenobiotic intake.

The present review has two objectives: (1) to provide an update on biliary physiology and xenobiotic excretion for forensic toxicology purposes; and (2) to serve as a practical tool at all levels of toxicological bile analysis: sampling, storage, analysis and, above all, interpretation. A Medline search was performed with the key-word "bile" combined to "forensic", "medicolegal", "quantification", "postmortem", "post mortem", "autopsy" and "chromatography + drug" (updated May 2015). The search was limited to the organic molecules most frequently encountered in routine forensic toxicology (some medical drugs, drugs of abuse and ethanol) and to publications after 1970.

2. Physiology

2.1. Anatomy of the liver and biliary system

The liver has a rich vascular system, comprising the hepatic artery, supplying highly oxygenated blood, and the portal vein, transporting nutrients from the digestive system. These vessels ramify in the liver lobules, which are functional units (Fig. 1), and join in the sinusoids draining into the central vein and then the systemic circulation via the hepatic veins [19]. With this double input, the liver purifies not only arterial blood, as do the kidneys, but also portal vein blood, eliminating toxins absorbed by the digestive system before they reach the general circulation: this is known as the first-pass liver effect. The liver sinusoids are fringed with hepatocytes, secreting bile into biliary canaliculi formed between two adjacent cells whose adjoining apical membranes are sealed by tight junctions [1]. These canaliculi are drained in ductules and then biliary canals which progressively join together in a complex network of intra- then extra-hepatic ducts (the biliary tree), to form the common hepatic duct. This then divides into the common bile duct or ductus choledochus, on the one hand, releasing bile into the duodenum via the sphincter of Oddi, and the cystic duct, on the other, feeding into the gallbladder where bile is

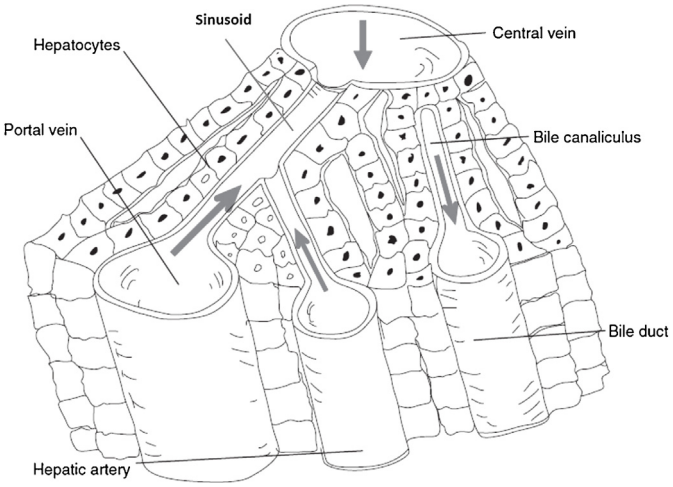

Fig. 1. Structure of a liver lobule. Adapted from [21] with permission. Portal vein and hepatic artery blood blends in the liver sinusoids, which drain into the central vein. Bile is secreted by hepatocytes, fringing the sinusoids, in the biliary canaliculi before entering the biliary ducts.

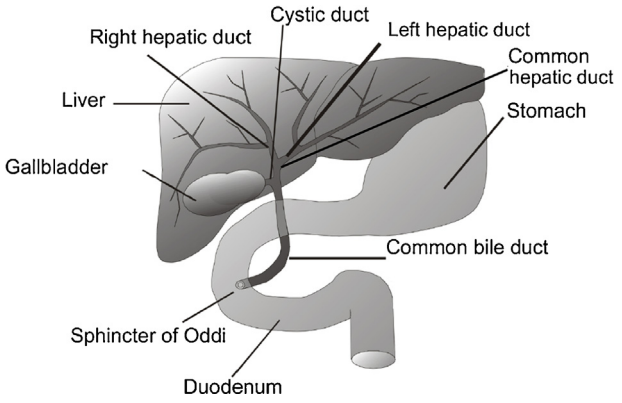

Fig. 2. Structure of the biliary tree.
Adapted from [18] with permission.

temporarily stored (Fig. 2) [20]. The gallbladder is a closed sac of about 50 mL capacity [21,22] fulfilling two main functions: concentrating bile, and storing it for release at the right moment during digestion. The gallbladder wall comprises a serous external layer, an intermediate muscular layer providing motility, and an epithelial layer of cells able to absorb water and various electrolytes and cells secreting a mucous protection against bile salts [23].

2.2. Bile formation

Hepatocytes secrete what is known as hepatic or canalicular bile. Bile flow results from complex processes of secretion (bile salts, phospholipids, vitamin D, etc.) and of excretion of substances to be eliminated (bilirubin, xenobiotics, etc.). Active transport of solutes, including bile salts (bile-salt-dependent bile flow) and other osmotically active compounds (bile-salt-independent bile flow), induces an osmotic gradient which in turn induces flow of water and electrolytes [1,24,25]. The bile is then modified by the bile duct wall cells (cholangiocytes), by absorption/secretion adjusting bile flow, bile-salts concentration and alkalinity [20]. The volume of bile produced by the liver is about 600 mL to 1 L per day, and is regulated by several signals such hormone release following a meal (secretin) and circulating bile salt level [1,18,24]. Outside of mealtimes, the sphincter of Oddi is closed and bile passes into the gallbladder, where it is stored and modified by reabsorption of water and electrolytes, resulting in gallbladder bile, which is 5–20 times as concentrated as canalicular bile [21,22]. In reaction to neural and hormonal (cholecystokinin) stimuli triggered by feeding, the gallbladder is partially emptied by muscle contractions and the bile is released into the intestine, where it facilitates absorption, notably of lipid and lipophilic substances. Gallbladder emptying is proportional to the lipid content of the meal [2]. During the meal, canalicular bile is delivered directly to the intestine but is stored in the gallbladder between two meals and during fasting [22,24]. Secretion is altered by liver pathology and by specific biliary pathway pathologies such as cholestasis or cholecystitis, especially those secondary to biliary tract lithiasis or cancer [5,23,26].

2.3. Bile composition

Canalicular bile is a yellow liquid, while gallbladder bile is thicker and deep yellow to green in color. Both are slightly alkaline. Bile is mainly constituted of water (95%) with compounds in solution or in suspension: electrolytes (sodium, potassium, calcium, chloride, bicarbonate), bile salts (particularly cholic and chenodeoxycholic acid), phospholipids (mainly lecithin), cholesterol, bile pigments (bilirubin, porphyrin, etc.) and proteins (immunoglobulins, albumin, etc.) [1]. Bile may thus be defined as a complex alkaline aqueous medium containing most of the solutes found in blood and micelles formed by bile salts able to contain the most lipophilic compounds. Table 1 presents the main components of canalicular and gallbladder bile.

Table 1
Main components of canalicular and gallbladder bile [22].

	Canalicular bile	Gallbladder bile
Water	97.5 g/dL	92 g/dL
Bile salts	1.1 g/dL	6 g/dL
Bilirubin	0.04 g/dL	0.3 g/dL
Cholesterol	0.1 g/dL	0.3–0.9 g/dL
Fatty acids	0.12 g/dL	0.3–1.2 g/dL
Lecithin	0.04 g/dL	0.3 g/dL
Na^+	145 mEq/L	130 mEq/L
K^+	5 mEq/L	12 mEq/L
Ca^{++}	5 mEq/L	23 mEq/L
Cl^-	100 mEq/L	25 mEq/L
HCO_3^-	28 mEq/L	10 mEq/L

2.4. Principle of biliary excretion of xenobiotics

Biliary excretion is one of the organism's means of eliminating exogenous substances [5], thereby contributing, along with metabolism, to the purification function of the liver. Biliary excretion of xenobiotics by hepatocytes requires two cell membranes to be crossed: the basolateral membrane of hepatocytes at the blood sinusoids side, and the apical membrane forming the biliary canaliculus (Fig. 3). Crossing of molecules, whether or not bound to plasma protein, from the sinusoid to the hepatocytic extracellular space, known as the space of Disse, is unrestricted, due to the fenestration of the epithelial cells (sinusoidal cells) and the lack of basal membrane in this type of blood capillary [27]. From the space of Disse, only the free fraction of the substance can penetrate the hepatocyte, by simple diffusion and/or active transfer; the molecule may then bind to intracellular protein, excrete back into the blood-flow or in the biliary canaliculi, either unmodified or in the form of metabolites. For some compounds, such as plasma proteins, other biliary excretion mechanisms have been described, such as endocytosis-exocytosis [24] or diffusion through the inter-hepatocyte tight junctions, which does not require cell penetration (para-cellular route) [1]. As mentioned above, the bile secreted by the hepatocyte is drained into the biliary system and then released into the duodenum, most of its components being eliminated in feces. Certain molecules, however, may be reabsorbed in the intestine; either in the form in which they were secreted (parent or metabolite) or after being metabolized by the intestinal flora, as in the case of glucuronide conjugates, where the parent molecule is reabsorbed following the action of intestinal glucuronidases. In molecules of forensic interest, such as morphine, colchicine, lorazepam or digoxin, enterohepatic cycle is well-known and results in systemic recirculation that may give rise to several plasma concentration peaks and lengthened elimination half-life [4,5].

2.5. Role of transporters

Xenobiotic penetration in hepatocytes may be by passive diffusion or by active transfer via a transporter; generally, the higher the molecular weight and/or hydrophilicity of the compound, the more its diffusion depends on active transport [18]. Due to the importance of the liver in metabolism and elimination, hepatocytes express numerous transporters able to

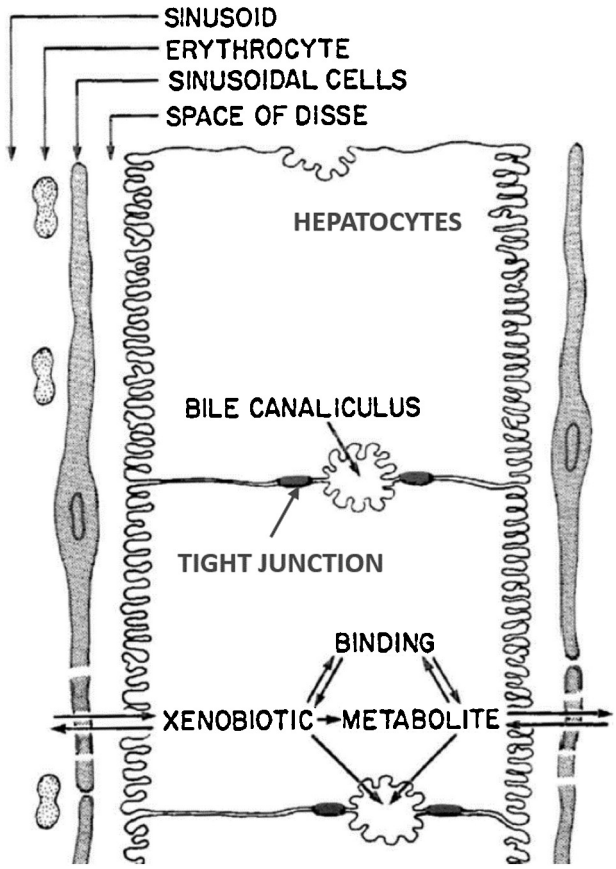

Fig. 3. Xenobiotic exchanges in the hepatocyte. Adapted from [278] with permission. The xenobiotic, circulating in the sinusoids, freely enters the space of Disse by intercellular gaps between endothelial cells, fenestrations, and lack of complete basal lamina. Then it penetrates the hepatocyte by simple diffusion and/or active transfer. Within the hepatocyte, it may bind to intracellular protein, excreted into bile or transferred back to the blood in parent or metabolite form.

bind circulating molecules. They comprise mainly two types of transmembrane protein: influx pumps of the SLC (SoLute Carrier) superfamily, and efflux pumps of the ABC (ATP Binding Cassette) superfamily [1]. Unlike passive diffusion, active transport may be limited by saturation if molecule concentration exceeds transporter capacity, by competition with other compounds or by inhibition by specific substrates [28].

Biliary excretion mainly depends on active transport of solutes essential to the composition and flow of bile. Concentrations that may be 10 to 100-fold higher than in blood require diffusion against the concentration gradient: active ATP-dependent transport is necessary [19,24,29,30] and passive diffusion may be considered negligible. Fig. 4 shows the main transporters found on hepatocyte membranes and their corresponding substrates.

2.6. Characteristics of bile-excreted molecules

As bile is difficult to analyze at the various stages of its formation, data for the characteristics of bile-excreted molecules are sparse and often derived from animal, in vitro models or in vivo in subjects with biliary pathology. Nevertheless, certain interesting facts can be highlighted. Molecules found in bile, whether endogenous or exogenous, can be divided into two groups according to plasma/bile concentration ratio: those with ratios less than or close to 1, and those with ratios much greater than 1 [3,16].

Ratios less than or close to 1 may correspond to poor hepatocyte absorption or to poor excretion into the biliary canaliculi (e.g., passive diffusion only) and/or subsequent reabsorption in the bile ducts or gallbladder [31] (as demonstrated for glucose [1] and methylmercury [32]). Moreover, certain strongly metabolized molecules have low bile clearance in their unmodified form whereas their metabolites may show non-negligible biliary excretion (morphine glucuronides [5]). Inter-subject metabolic differences, whether pathologic or genetic, may thus affect bile clearance of xenobiotics [26,33,34].

Molecules with elevated bile excretion have been the focus of several studies. In the 1970s, a molecular weight threshold was determined below which compounds are unlikely to be excreted in

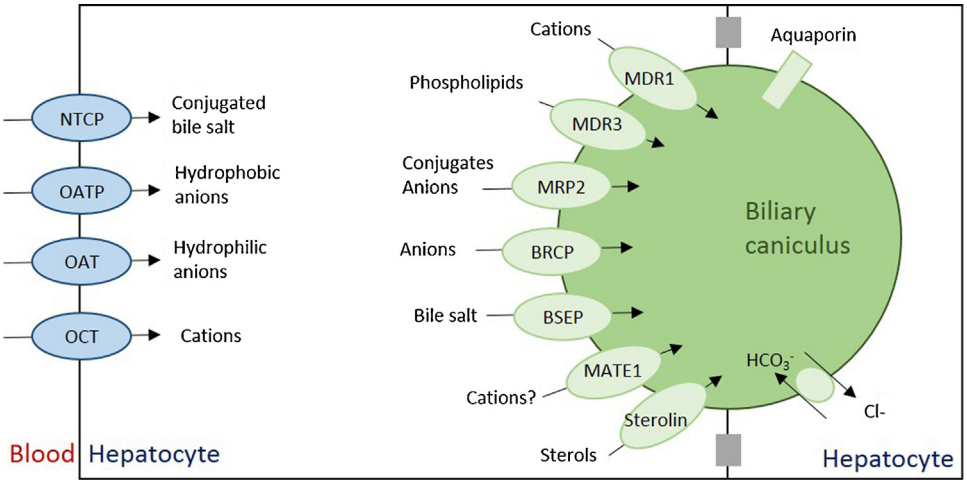

Fig. 4. Membrane transporters that determine the uptake and excretion of organic solutes in hepatocytes.
Uptake from blood (basolateral membrane) [1]:-. NTCP (sodium taurocholate cotransporting polypeptide): primary carrier for conjugated bile salt uptake.-. OATP (organic anion transporting polypeptides): broad substrate carriers of large hydrophobic anions and amphipathic organic solutes such as bile salt.-. OAT (organic anion transporter): affinity for hydrophilic anions.-. OCT (organic cation transporters): sodium independent uptake of small organic cations.
Excretion in bile (canalicular membrane) [1]:-. MDR1 (multidrug resistance 1, P-glycoprotein): ATP dependent excretion of organic cations.-. MDR3 (multidrug resistance 3): affinity for phospholipids.-. MRP2 (multidrug resistance associated protein 2, previously known as multispecific organic anion transporter MOAT): affinity for multivalent anions or amphipathic solutes such as conjugated metabolite.-. BRCP (breast cancer resistance protein): substrate overlap with MRP2, multispecific anions transporters.-. BSEP (bile salt export pump): affinity for bile salt.-. MATE1 (multidrug and toxin extrusion transporter): organic cation/H^+ exchanger such as cationic xenobiotic.-. Steroline 1 et 2 (ABCG5 and ABCG8 gene): affinity for cholesterol and other sterols.-. Chloride/bicarbonate exchanger.-. Aquaporine: water diffusion.

bile, at around 325 g/mol in rat and 500–600 g/mol in humans [3,5,35,36]. Lipophilicity was also identified as a determining factor. A study of endogenous and exogenous steroid hormones tended to show that more apolar compounds (e.g., progesterone) showed greater bile excretion than more polar compounds (e.g., hydrocortisone) [5]. A similar tendency was also found for cations such as triethylmethylammonium, tripropylmethylammonium, thiazinium, etc. [5]. A rough-and-ready idea thus came to be adopted that small polar molecules are eliminated via the kidneys and "the others" via biliary/fecal routes. More recent studies showed that molecular weight and lipophilicity are not enough to predict bile concentrations. More complex QSPKR (quantitative structure-pharmacokinetic parameters relationship) or PBPK (physiologically based pharmacokinetic) models were described, based, for example, on physicochemical properties: steric bulk, permeability and solubility, and polarizability [29,35,36]. Another determining factor in biliary excretion was reported to be affinity for transporters located on hepatocyte sinusoid and canaliculus membranes (Fig. 4). As mentioned above, active transport plays a preponderant role. Transporter substrate and cell expression are increasingly the focus of studies. These studies, however, mainly concern endogenous substrates, inhibitors involved in drug interactions or certain therapeutic classes such as anticancer, antibiotic and antiviral [1,24,27,29,37], and few focused on molecules of forensic interest. For example, glycoprotein P (P-gp or MDR) has been the focus of many studies concerning its functions [38,39] and role in biliary transport [1,25]. It is notably involved in extracellular transport of endogenous and exogenous organic cations [4] but only a few forensic compounds are identified as its substrate (e.g., verapamil [1], digoxin [40], certain benzodiazepines [41] and alkaloids [4]).

2.7. Postmortem evolution of bile

To the best of our knowledge, no data are available for the postmortem evolution of bile and its availability at autopsy. Availability may depend on how full the gallbladder is and thus on time of death with respect to mealtime. Moreover, bile may be subject to postmortem processes which could probably affect composition: perivascular diffusion following gallbladder damage, or dehydration. In the Forensic Medicine Institute of Lyon (France), bile could be sampled in 85% of autopsies performed between 2010 and 2013 (personal data).

3. Bile analysis

3.1. Sampling and storage

During autopsy, bile can easily be collected by syringe aspiration from the gallbladder, or by incision-compression if the bile is too viscous [42]. The entire content or up to 10–15 mL should be sampled [42–45]. The container should be adapted to the volume, to avoid evaporation of volatile molecules in the head space, oxidation or possible precipitation if a preservative agent is used in overconcentration [46]. In case of cholecystectomy, sampling is trickier, but can be performed directly in the common bile duct [43]: the toxicologist should be informed of the sampling site, given the differences in composition between liver and gallbladder bile. Some authors recommend collecting bile ahead of organ dissection, to avoid contamination both of the bile, notably by stomach or intestine contents, and of the organs by the bile [43,47]. For the same purpose, ligating the gallbladder ahead of sampling is also recommended although the efficacy of this measure has not, to our knowledge, been assessed [45,46,48].

There have been few studies focusing on the stability of bile samples according to storage conditions. To prevent hydrolysis of glucuronide metabolites, the sample can be slightly acidified by adding a buffer (e.g. ammonium acetate 1 M pH 4.0/5.5 or sodium acetate 0.1 M pH 5) or acid (acetic or chlorhydric) at the moment of sampling [49,50]. Melo et al. [51] used liquid chromatography coupled to a ultraviolet (UV) detector to study the bile stability of four benzodiazepines (lorazepam, estazolam, ketazolam and chlordiazepoxide) up to 6 months at −80 °C, −20 °C, +4 °C and +25 °C with or without addition of sodium fluoride (NaF) as preservative. Sampling with preservative, storage at 4 °C for a short period and freezing for long-term storage were recommended, to limit degradation of these benzodiazepines in bile. Chlordiazepoxide stability was better in bile than in blood, which the authors attributed to bile being a relatively unfavorable environment for the development of microorganisms. In the absence of specific data on bile samples, guidelines for storage of specific molecules obtained from other matrices are relevant.

3.2. Analytic procedures

3.2.1. Screening

Immunologic screening for certain groups of xenobiotics is feasible in bile [52,53]. The immuno-analysis kit may, however, require pre-treatment by solvent or protein precipitation [53] to reduce viscosity and matrix interference. The Abbott TDx analyzer, no longer on the market, was based on fluorescence polarization immuno-analysis (FPIA). It was assessed for opiate screening in bile [54]: detection required adapting the protocol, initially designed for blood, by increasing the dilution to reduce the intensity of matrix fluorescence; quantification required using a standard addition method, as the matrix effect varied from sample to sample. Other immunologic techniques have been used to screen for opiates [55,56], cocaine derivatives [57,58], acetaminophen [17] and alcohol [59] in bile.

The first reported general unknown screening (GUS) for medical drugs and narcotics specifically in bile consisted in liquid/solid extraction after lyophilization associated to thin layer chromatography [60]. The automated REMEDI (Rapid EMErgency Drug Identification) system, which is no longer on the market, was assessed for GUS in a forensic context, in blood and alternative matrices including bile [61]. Vanbinst et al. [17] described a screening method common to blood, urine, bile and stomach contents, based on liquid/liquid extraction (LLE) under acid and alkaline pH and extract analysis by high performance liquid chromatography (HPLC) with diode array detector (DAD) and gas chromatography (GC) coupled to mass spectrometry (MS). For the same four matrices, Soriano et al. [62] developed a targeted screening method, assessed for 25 molecules, using solid phase extraction (SPE) coupled to GC/MS and GC with nitrogen phosphorus detector (NPD), with special attention given to the development of the extraction. Bevalot et al. [63] described an SPE method with double elution as a basis for developing GUS or targeted analyses in alternative matrices; it was validated for bile on 6 molecules of varied pharmacokinetic properties (morphine, 6-MAM, meprobamate, cyamemazine and caffeine) and assessed on 12 others of forensic interest.

3.2.2. Specific assay methods

Pharmacokinetic studies require high reliability; therefore bile-specific analytic procedures have been developed and validated [49]. For example, analysis of cannabinoids and certain of their conjugated metabolites was validated in bile for the study of biliary excretion [64]. In forensic toxicology, a few analytic methods have been adapted and/or validated specifically for quantification in bile; for example, the literature mentions assays for morphine [65,66], cocaine [57], 4 benzodiazepines associated to citalopram [67] and 5 combined molecules (morphine, 6-MAM, meprobamate, cyamemazine and caffeine) [63].

Bile, however, entails certain analytic problems due to its complex composition. It is an excretion medium for high-concentration endogenous and exogenous compounds or phase-1 metabolites structurally close to their parent molecule [49,68]. This may induce interferences affecting sensitivity and specificity, especially when an insufficiently specific detector, such as by UV or flame ionization, is used [66]. In mass spectrometry, interferences may not be visible but still induce a matrix effect reducing or boosting the ion signal and impairing reliability [49]. Fabritius et al. [64] reported 20–80% signal suppression in an LC/MSMS assay method for various bile cannabinoids; however, the ion suppression effect did not impact the accuracy of the test. In contrast, Lauer et al. [69] developed a colchicine quantification method in matrices collected and dried on filter paper (DBS: dried blood spot technique). Successfully validated in blood and 8 other fluids and biological tissues, it did not enable reliable quantification of colchicine in bile due to a major matrix effect with 99.3% signal suppression. Thus, this filter-paper method, consisting in a simple methanol extraction, is an inadequate purification technique for the complexity of bile. The authors concluded by citing Skopp's rule [42] that it is better not to transfer methods validated on other biological fluids directly to bile. Sensitivity is not usually the focus of bile analysis, as concentrations tend to be high [53,66,68], and it is better to have an effective purification technique, ensuring better specificity and reliability even with a lower level of extraction.

Despite the above problems of bile analysis, the literature is rich in case reports describing xenobiotic distribution in biological matrices, including bile, in which the analytic technique is transferred directly from that used for blood or adapted without assessment. This may be suitable for a single case report aimed at comparing concentrations between matrices and considering the uncertainties relating to postmortem phenomena (redistribution, stability, etc.) [49]. The standard addition method may provide a good solution, taking account of the nature of the matrix, but requires a sufficient sample quantity [17]. To improve assay quality, calibration in blank bile should be used with concentrations adapted to bile, as well as an internal standard structurally close to the analyte, such as a deuterated analog if available. For larger series, Hoizey et al. [70] assessed the reliability of a method transferred to another matrix by performing quality controls prepared by doping matching blank matrix. Finally, for more systematic use or pharmacokinetic study, validation should be performed following current guidelines such as those of the Scientific Working Group for Forensic Toxicology [71]. In the case of analytical assays intended for several matrices, crossed validation is a satisfactory means of ensuring reliability with a limited number of trials; it meets Food and Drug Administration guidelines [72] and was applied for partial validation of a bile analysis method based on a method entirely validated in blood [69] or in bone marrow [63,67]. The validation criteria for partial validation may include the following: selectivity, limit of quantification, range linearity, accuracy and intermediate precision at several concentration levels.

3.2.3. Free and conjugated forms: hydrolysis procedures

Like blood or urine, bile can be hydrolyzed to quantify both free and conjugated forms of molecules. Various enzymatic procedures were described to estimate total morphine in bile: *Patella vulgata* glucuronidase (3000–5000 U, overnight incubation at 55 °C) [66], β-glucuronidase at pH 5.1 [73], *Escherichia coli* β-glucuronidase (overnight incubation at 47 °C) [74]. Nakamura and Way [65] also employed chemical hydrolysis to quantify free plus conjugated morphine: bile samples were treated with 1 N sulfuric acid and autoclaved for 15 min at 15 pounds. Free and conjugated forms of benzodiazepines were evaluated in rat bile by St-Pierre and Pang [75] by enzymatic hydrolysis (*Helix pomatia* β-glucuronidase, 500 U, at pH 5, incubation 24 h at 37 °C). These studies show that classical hydrolysis procedures can be employed with success on bile samples.

4. Interpretation of bile analysis results

Bile concentrations are reported in many case reports, usually alongside assays in others matrices and without discussion. There are few large autopsy series or animal studies focusing on the advantages and drawbacks of bile as study matrix in forensic toxicology.

4.1. Case reports

Table 2 presents the case reports retrieved in the literature referenced in Medline which report bile concentrations, with the number of cases, cause of death and blood concentration. Bile/blood concentration ratios were calculated from these data. It is intended as a working tool for toxicologists analyzing and interpreting particular molecules. The higher concentrations in bile than blood have often been highlighted. This can be objectified from the numerous case reports in Table 2, since more than 80% of the 135 compounds presented bile/blood concentration ratios clearly greater than 1. Many of them are of interest forensic toxicology, such as narcotics, toxic principles of plant origin, psychotropic drugs, etc.

4.2. Qualitative interest

The higher concentrations in bile as compared to blood have often been highlighted. The extent of bile excretion can be seen from significant autopsy series for most narcotics: opioids (morphine and codeine) [66,73], fentanyl [76], oxycodone [77], buprenorphine [78], amphetamine derivatives [79], cocaine derivatives [16,57] and cannabinoids [80].

Bile excretion of morphine and morphine glucuronide conjugates has been widely described and the existence of enterohepatic circulation [4,5,19,81] may induce prolonged presence in blood and bile. Buprenorphine showed massive bile excretion as parent molecule (mean bile/blood ratio of 9638, $n = 12$) or as the metabolite norbubrenorphine [78]. For cannabis, the bile/blood concentration ratio is low for Δ-9-tétrahydrocannabinol (THC) but higher for its metabolites, including 11-Nor-9-carboxy-THC (THC-COOH), 11-hydroxy-THC (11OH-THC), and the THC and THC-COOH glucuronides [64,82]. In a series of 50 road accident deaths involving cannabis [80], the bile/cardiac blood concentration ratio of total THCCOOH (free + glucuronide) ranged from 17.2 to 888; THC was not analyzed. Like morphine, the presence in gallbladder of THCCOOH glucuronides, liable to be hydrolyzed by intestinal flora and reabsorbed, suggested enterohepatic recirculation that could contribute to the long detection time of cannabinoids [3]. Two studies compared bile and blood concentrations for cocaine derivatives. The first, using radio-immunologic assay, reported ratios ranging from 2.15 to 107.53 (mean = 30.79) over 20 cases [57]. The second, in a series of 50 cases (analytic method not specified), found ratios between 5 and 7 for cocaine and its metabolites [16]. Bile excretion levels are lower for amphetamine derivatives. Liu et al. [79] reported a bile/blood ratio of 0.64 to 9.8 for 3,4-methylenedioxy-methamphetamine (MDMA) ($n = 11$). Vanbinst et al. [17] reported ratios of 1.19 for amphetamine ($n = 1$) and 1.59 for MDMA ($n = 2$); these were among the lowest ratios in this study of 36 medicines or drugs of abuse. In this study, drugs of abuse (cocaine, 2-ethylidine-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP), MDMA, methadone, morphine and codeine) were detected in bile but not blood in 36.3% of cases ($n = 44$). Agarwal and Lemos [16] likewise found that in several cases cocaethylene was detectable only in bile.

Table 2
Case reports providing bile concentrations: substance name, number of cases reported (*n*), cause of death, blood concentrations (peripheral and/or cardiac, "blood" if not specified), bile concentration.

Substance	*n*	Cause of death	Blood concentration		Bile concentration (range)	Ratio Bile/PB & Bile/CB	Ref.
			Peripheral blood	Cardiac blood			
25I-NBOMe	1	*Fatal intoxication involving 25I-NBOMe*	405 pg/mL	410 pg/mL	10.9 ng/g	#/#	[111]
3,5-dimethoxyphenol	1	*Fatal poisoning by Taxus*	217 ng/mL	100 ng/mL	175 ng/mL	0.81/1.8	[112]
4-MTA[a]	1	*Overdose fatality involving 4-MTA and MDMA*[a]	5.49 mg/L	7.60 mg/L	36.4 mg/L	6.6/4.8	[113]
6-MAM[a]	1	*Multiple drug intoxication*	Blood: 0.93 ng/mL		ND	–	[114]
Acepromazine	1	*Suicide by acepromazine intoxication*	Chest cavity blood: 0.6 μg/mL		6.5 μg/mL	10	[115]
Acepromazine	1	*Suicide by acute zolpidem overdose*	Blood: 2.4 mg/L		1.03 mg/L	0.43	[116]
Acetaminophen	1	*Multiple drug intoxication*	Left femoral vein: 60 mg/L Right femoral vein: 60 mg/L	Thoracic: 57 mg/L	73 mg/L	1.2/1.3	[117]
Acetaminophen	3	*Suicidal poisoning by co-proxamol*	414 mg/L 261 mg/L 184 mg/L	384 mg/L 345 mg/L 178 mg/L	668 mg/L 598 mg/L 676 mg/L	1.6/1.7 2.3/1.7 3.7/3.8	[118]
Aconitine	2	*Suicidal Aconitum poisoning*	10.3 μg/L 15.4 μg/L	14.9 μg/L 33.7 μg/L	139 μg/L 240 μg/L	14/9.3 16/7.1	[119]
Aconitine	1	*Suicide by ingestion of aconite*	Serum: 1.1 ng/mL		6.3 ng/g	#	[120]
AH-7921[a]	1	*Accidental opioid intoxication*	9.1 mg/L	3.9 mg/L	17 mg/L	1.9/4.4	[121]
Alimemazine	1	*Fatal intoxication involving alimemazine*	Blood: 6.52 μg/mL		4.44 μg/mL	0.68	[122]
Alpha-methylfentanyl	1	*Overdose by* alpha-methylfentanyl	Blood: 3.1 ng/mL		6.4 ng/mL	2.0	[123]
Alprazolam	1	*Suicide by acute alprazolam overdose*	2.3 mg/L	2.1 mg/L	2.8 mg/L	1.2/1.3	[124]
Amitriptyline	1	*Fatal self-poisoning involving amitriptyline*	Blood: 0.82 mg/L		8.01 mg/L	9.8	[125]
Amitriptyline	1	*Drug-related death*	Blood: 0.40 μg/mL (Nortriptyline: 1.32 μg/mL)		13.16 μg/mL (Nortriptyline: 11.62 μg/mL)	33 (Nortriptyline 8.8)	[126]
Amoxapine	3	*Suicide by amoxapine overdose*	Blood: 11.5 mg/L Blood: 2.80 mg/L Blood: 0.89 mg/L		1264.5 mg/L 69.1 mg/L 14.30 mg/L	110 25 16	[127]
Amoxapine	2	*Suicide by multiple drug intoxication*	Blood: 18 ± 2.2 mg/L Blood: 6.7 ± 0.9 mg/L		61 ± 11 mg/L ND	3.4 –	[128]
Amphetamine	1	*Suicide by methamphetamine overdose*	0.74 mg/L	–	0.72 mg/L	1.0/–	[129]
Atomoxetine	2	*–Arrhythmogenic right ventricular dysplasia* *–Suicide by venlafaxine and atomoxetine overdose*	0.33 mg/L 5.4 mg/L	0.65 mg/L 8.3 mg/L	1.0 mg/L 33 mg/L	3.0/1.5 6.4/1.2	[130]
Brucine	1	*Suicide by brucine overdose*	1.51 μg/mL	–	9.94 μg/mL	6.6/–	[131]
Buflomedil	1	*Fatal intoxication involving buflomedil*	–	24.5 μg/mL	39.1 mg/mL	–/1.6	[132]
Buformin	1	*Lactic acidosis*	Serum: 3.2 mg/L		6.3 mg/L	2.0	[133]
Buprenorphine	1	*Suicide by buprenorphine intoxication*	Blood: 3.3 mg/L		2035 mg/L	616	[134]
Buprenorphine	13	*Fatal intoxication involving buprenorphine*	Blood: 6.12 ng/mL (1.1–18.0)		23,852 ng/mL (575–72650)	3897	[78]

Table 2 (*Continued*)

Substance	*n*	Cause of death	Blood concentration		Bile concentration (range)	Ratio Bile/PB & Bile/CB	Ref.
			Peripheral blood	Cardiac blood			
Buprenorphine Norbuprenorphine (Nor-)	1	*Polyintoxication with rapid onset*	Blood: Free:2.35 ng/mL Conjugated: ND Nor-(free): 0.15 ng/mL		Free: 150 ng/mL Conjugated: 850 ng/mL Nor-(free): ND	Free: 64 Conjugated: – Nor-(free): –	[135]
Bupropion	1	*Fatal intoxication by bupropion and ethanol*	Subclavian: 6.2 mg/L	–	1.4 mg/L	0.23/–	[136]
Caffeine	4	*–Suicide by overdose*	Blood: 147.0 mg/L		64.3 mg/L	0.44	[137]
		–Suicide by overdose	Blood: 343.9 mg/L		263.2 mg/L	0.77	
		–Suicide by overdose	Blood: 251.0 mg/L		312.0 mg/L	1.2	
		–Accidental Fatal overdose	Blood: 184.1 mg/L		200.2 mg/L	1.1	
Carbamazepine	1	*Multiple drug intoxication*	Blood: 79 mg/L		69 mg/L	0.87	[138]
Chlorprothixene	1	*Fatal intoxication involving chlorprothixene*	Blood: 0.10 mg/L		3.9 mg/L	39	[139]
Citalopram	7	*–Fatal intoxication involving citalopram*	0.8 mg/L	–	6.0 mg/L	7.5/–	[140]
		–Multiple drug intoxication	0.4 mg/L (0.2–0.7)	–	2.77 mg/L (0.8–4.3)	6.9/–	
		–Other	0.3 mg/L (0.2–0.4)	–	1.63 mg/L (1.3–2.1)	5.4/–	
Citalopram	1	*Fatal intoxication involving topiramate*	Blood: 0.85 mg/L		7.3 mg/L	8.6	[141]
Citalopram	1	*Suicide by citalopram and cyproheptadine intoxication*	2.3 mg/L	–	9.0 mg/L	3.9/–	[142]
Clomipramine	1	*Drug-related death*	Blood: 2.86 μg/mL		19.70 μg/mL	6.9	[126]
Clotiapine	4	*–Acute mixed intoxication*	110 μg/L	75 μg/L	657 μg/L	6.0/8.8	[143]
		–Not known	310 μg/L	–	1860 μg/L	6.0/–	
		–Acute mixed intoxication	340 μg/L	200 μg/L	6320 μg/L	19/32	
		–Not known	58 μg/L	50 μg/L	9120 μg/L	157/182	
Clozapine	1	*Suicide by acute clozapine overdose*	8.8 mg/L	12.0 mg/L	1844 mg/L	209/154	[144]
Clozapine	1	*Suicide by multiple drug intoxication*	–	0.2 mg/L	NQ	–/–	[145]
Cocaine	4	*Fatal intoxication involving cocaine*	Blood: 6.9 mg/L		18.0 mg/L	2.6	[146]
			Blood: 3.9 mg/L		8.2 mg/L	2.1	
			Blood: 1.8 mg/L		10.0 mg/L	5.5	
			Blood: 13.0 mg/L		25.0 mg/L	1.9	
Cocaine	1	*Overdose fatality involving cocaine*	Blood: 330 mg/L		25 mg/L	0.07	[147]
Cocaine (BZE[a])	1	*Cocaine poisoning in a body packer*	4 μg/mL (BZE = 17.0 μg/mL)		99.8 μg/mL (BZE = 54.0 μg/mL)	25 BZE = 3.2	[148]
Cocaine	1	*Overdose by cocaine*	Blood: 51.7 mg/L		46.8 mg/L	0.91	[149]
Cocaine	2	*Overdose by cocaine*	Blood: 5 mg/L		1.5 mg/L	0.30	[150]
			Blood: 1.6 mg/L		2.0 mg/L	1.3	
Cocaine (BZE; EME[a])	1	*Cocaine overdose*	5.0 mg/L (BZE = 10.4 mg/L; EME = 4.1 mg/L)	9.0 mg/L (BZE = 20.1 mg/L; EME = 14.4 mg/L)	5.3 mg/L (BZE = 19.1 mg/L; EME = 6.2 mg/L)	1.0/0.59 BZE = 1.8/0.95 EME = 1.5/0.43	[151]
Cocaine	1	*Fatal intoxication involving cocaine*	–	–	0.75 μg/mL	–/–	[57]
Codeine	1	*Acute heroin fatality*	NA Embalmment		305 ng/mL	–	[152]
Codeine	3	*Multiple drug intoxication*	Blood: 17.2 ng/mL (7.9–25)		55.5 ng/mL (19.4–80.1)	3.2	[114]
Codeine	1	*Fatal intoxication involving codeine*	Whole Blood: 22.1 mg/L		9.2 mg/L	0.42	[153]
Codeine	1	*Possible overdose by heroin*	37 μg/L	–	88 μg/L	2.4/–	[154]

Codeine	1	*Multiple drug intoxication*	Right femoral vein: 0.33 mg/L Left femoral vein: 0.39 mg/L	Thoracic: 0.49 mg/L	0.84 mg/L	Right: 2.5 Left: 2.2/1.7	[117]
Colchicine	2	*Suicidal colchicine poisoning*	17.4 ng/mL 21.9 ng/mL	5.2 ng/mL 22.8 ng/mL	42.8 ng/mL 1818.5 ng/mL	2.5/8.2 83/80	[155]
Colchicine	1	*Fatal accidental intoxication by colchicine*	–	50 g/L	12,000 μg/L	–/240	[156]
Colchicine	1	*Suicide by colchicine intoxication*	–	66 ng/mL	5632 ng/mL	–/85	[157]
Colchicine	1	*Accidental intoxication by colchicine*	Blood: ND		7.5 μg/mL	–	[87]
Colchicine	1	*Suicide by colchicine poisoning*	62 ng/mL	–	2921 ng/mL	47/–	[86]
Cyproheptadine	1	*Suicide by citalopram and cyproheptadine intoxication*	0.49 mg/L	–	30.7 mg/L	62/–	[142]
Cyproheptadine	1	*Fatal intoxication involving ethanol and cyproheptadine*	0.47 mg/L	0.62 mg/L	8.1 mg/L	17/13	[158]
Cytisine	1	*Fatal intoxication involving cytisine*	2.5 ng/mL	0.9 ng/mL	6.1 ng/mL	2.4/6.7	[159]
Desipramine	1	*Multiple drug intoxication*	Blood: 4.2 mg/L		23 mg/L	5.5	[138]
Despropionyl Bezitramide	1	*Multiple drug intoxication*	Blood: 106.1 ng/mL		621.5 ng/mL	5.9	[160]
Dextromoramide	2	*Fatal overdose involving dextromoramide*	–	871.1 ng/mL	50.2 ng/mL	–/0.06	[161]
			–	984.3 ng/mL	175.0 ng/mL	–/0.17	
Diflunisal	1	*Fatal intoxication involving diflunisal*	Blood: 260 mg/L		71 mg/L	0.27	[162]
Digoxin	1	*Fatal intoxication involving digoxin and doxepin*	Blood: 169 ng/mL		4900 ng/mL	29	[163]
Diltiazem	1	*Acute intoxication involving diltiazem and pentoxifylline and cardiomyopathy*	Blood: 0.59 mg/dL		0.4 mg/dL	0.68	[164]
Diltiazem	1	*Fatal intoxication involving diltiazem*	Blood: 31.1 mg/L		294.9 mg/L	9.5	[165]
DMT[a]	1	*Hallucinogenic amine intoxication*	0.01 mg/L 5-methoxy-DMT: 1.20 mg/L	0.02 mg/L 5-methoxy-DMT: 1.88 mg/L	0.57 mg/L 5-methoxy-DMT: 9.81 mg/L	57/29 5-methoxy-DMT: 8.2/5.2	[166]
Diphenhydramine	1	*Multiple drug intoxication*	Right femoral vein: 0.41 mg/L Left femoral vein: 0.45 mg/L	Thoracic: 0.66 mg/L	2.3 mg/L	Right: 5.6 Left: 5.1/3.5	[117]
Disopyramide	1	*Accidental overdose of disopyramide and sulindac*	Blood: 41.3 mg/L		435 mg/L	10	[167]
Dizocilpine (MK-801)	1	*Multiple drug intoxication*	Blood: 0.15 mg/L		0.29 mg/L	1.9	[168]
Dothiepin	1	*Overdose by dothiepin*	Blood: 5.75 mg/L		110 mg/L	1.9	[169]
Doxacurium	1	*Fatal intoxication of doxacurium*	–	>LOD (10 ng/mL) NQ	> LOD (10 ng/mL) NQ	–/–	[170]
Doxepin	1	*Fatal intoxication involving digoxin and doxepin*	Blood: 1.8 mg/L		15 mg/L	8.3	[163]
Doxepin	7	– *Acute fatal doxepin intoxication*	780 ng/mL	998 ng/mL	110,924 ng/mL	142/111	[171]
		– *Acute fatal doxepin intoxication*	3786 ng/mL	4111 ng/mL	101,553 ng/mL	27/25	
		– *Multiple drug intoxication*	113 ng/mL	139 ng/mL	1510 ng/mL	13/11	
		– *Multiple drug intoxication*	34.4 ng/mL	45.4 ng/mL	778 ng/mL	22/17	
		– *Multiple drug intoxication*	84.0 ng/mL	106 ng/mL	1220 ng/mL	14/12	
		– *Other*	378 ng/mL	549 ng/mL	35,997 ng/mL	95/66	
		– *Unknown*	41.4 ng/mL	45.9 ng/mL	1310 ng/mL	31/28	
Duloxetine	7	– *Multiple drug intoxication*	0.10 mg/L	0.17 mg/L	1.1 mg/L	11/6.5	[172]
		– *Multiple drug intoxication*	0.09 mg/L	0.22 mg/L	2.0 mg/L	22/10	
		– *Multiple drug intoxication*	–	0.28 mg/L	1.3 mg/L	–/4.6	
		– *Morphine intoxication*	–	0.22 mg/L	1.3 mg/L	–/5.9	
		– *Poly-med overuse*	0.26 mg/L	0.59 mg/L	3.1 mg/L	12/5.3	

Table 2 (*Continued*)

Substance	*n*	Cause of death	Blood concentration		Bile concentration (range)	Ratio Bile/PB & Bile/CB	Ref.
			Peripheral blood	Cardiac blood			
		– *Arteriosclerotic cardiovascular disease*	0.05 mg/L	0.08 mg/L	0.57 mg/L	11/7.1	
		– *Methadone intoxication*	0.20 mg/L	0.23 mg/L	0.69 mg/L	3.5/3.0	
Ethyltryptamine	1	*Fatal intoxication involving ethyltryptamine*	–	5.6 mg/L	22.0 mg/L	–/3.9	[173]
Etomidate	3	– *Suicide by etomidate intoxication*	0.40 mg/L	–	0.46 mg/L	1.2/–	[174]
		– *Medical intervention/Crush injuries*	0.05 mg/L	–	0.37 mg/L	7.4/–	
		– *Medical intervention/injury at chest and abdomen*	<0.026 mg/L	–	0.11 mg/L	–/–	
Fentanyl	15	– *Fatal intoxication involving fentanyl*	11.6 μg/mL (4.5–18)	22.2 μg/mL (3.5–55)	103.3 μg/mL (3.5–197)	8.9/4.7	[76]
		– *Other*	11.2 μg/mL (6.8–19)	17.9 μg/mL (+<2.0–81)	57.7 μg/mL (5.9–262)	5.1/3.2	
		– *Suicide by multiple drug intoxication*	–	Embalmed	25 μg/L	–/–	
Fentanyl	1	*Overdose by fentanyl*	Blood: 27.5 μg/L		58.2 μg/L	2.1	[175]
Fentanyl	1	*Suicide by acute fentanyl intoxication*	Right femoral vein: 21.3 μg/L Left femoral vein: 20.9 μg/L	Right chamber: 37.6 μg/L Left chamber: 33.9 μg/L	122.5 μg/L	Right:5.8/3.3 Left:5.9/3.6	[176]
Fentanyl	1	*Fatal intoxication involving fentanyl*	Blood: 4.8 μg/L		4.7 μg/L	0.98	[177]
Fentanyl	1	*Combined intoxication with fentanyl and bromazepam*	60.6 μg/L	94.1 μg/L	436.4 μg/L	7.2/4.6	[178]
Fentanyl	1	*Fatal intoxication involving fentanyl*	Blood: 4.9 ng/mL		8.8 ng/mL	1.8	[179]
Fentanyl	1	*Suicidal intoxication by fentanyl*	94.9 ng/g	Right heart:74.8 ng/g Left heart: 45.9 ng/g	468 ng/g	4.9/Right:6.3 Left:10	[180]
Flecainide	1	*Fatal intoxication involving flecainide*	Blood: 13 mg/L		160 mg/L	12	[181]
Flecainide	1	*Suicide by flecainide intoxication*	Blood: 93.7 mg/L		418.9 mg/L	4.5	[182]
Flecainide	1	*Multiple drug intoxication*	7.7 mg/kg	–	0.26 mg/kg	0.03/–	[183]
Fluoxetine	8	*Civil aviation accident*	Blood: 0.35 μg/mL (0.021–0.682)		3.51 μg/mL (0.126–5.90)	10	[184]
Fluoxetine	1	*Suicide by fluoxetine intoxication*	Blood: 6000 ng/mL		13,000 ng/mL	2.2	[185]
Flurazepam	1	*Suicide by acute flurazepam overdose*	5.5 mg/L	–	33 mg/L	6.0/–	[186]
Flurazepam	1	*Suicide by acute intoxication of flurazepam*	–	2.8 μg/mL	323 μg/mL	–/115	[187]
GHB[a]	1	*Fatal intoxication GHB/Heroin*	11.5 μg/mL		57.0 μg/mL	5.0	[188]
GHB[a]	1	*Fatal overdose involving GHB*	2937 mg/L	3385 mg/L	1800 mg/L	0.61/0.53	[189]
Haloperidol	1	*Suicide by multiple drug intoxication*	–	0.2 mg/L	NQ	–/–	[145]
Haloperidol	2	– *Suicidal overdose by haloperidol*	Blood: 1.9 mg/L		3.4 mg/L	1.8	[190]
		– *Natural cardiac death*	Blood: 0.6 mg/L		0.4 mg/L	0.67	
Halothane	2	– *Homicide by forced halothane inhalation*	Blood: 7.2 mg/kg		7.5 mg/kg	1.0	[191]
			Blood: 3.0 mg/kg		1.3 mg/kg	0.43	

Harmine	1	*Hallucinogenic amine intoxication*	0.08 mg/L Harmaline:0.04 mg/L Tetrahydroharmine: 0.24 mg/L		0.17 mg/L Harmaline: 0.07 mg/L Tetrahydroharmine: 0.38 mg/L	1.64 mg/L Harmaline: 0.41 mg/L Tetrahydroharmine: 4.78 mg/L	21/9.6 Harmaline: 10/5.8 Tetrahydroharmine: 20/13	[166]
Hydroxyzine	1	*Suicide by overdose of hydroxyzine*		Blood: 39 mg/L		122 mg/L	3.1	[192]
Hydroxyzine	1	*Fatal intoxication involving hydroxyzine*		Blood: 4.18 μg/mL		23.24 μg/mL	5.6	[193]
Ibogaine	1	*Fatal poisoning involving ibogaine*	5.4 ± 1.4 ng/mL		6.6 ± 0.6 ng/mL	21.3 ± 5.6 ng/mL	3.9/3.2	[194]
Imipramine	1	*Multiple drug intoxication*	Right femoral vein: 2.5 mg/L Left femoral vein: 2.3 mg/L		Thoracic: 5.2 mg/L	19 mg/L	Right:8.3/3.7 Left:7.6	[117]
Isoniazid	1	*Fatal intoxication involving isoniazid*	94 mg/L		43 mg/L	900 mg/L	9.6/21	[195]
Jesaconitine	1	*Suicide by ingestion of aconite*		Serum: 69.1 ng/mL		237.8 ng/g	#	[120]
Ketamine	1	*Homicide by ketamine intoxication*		Blood: 27.4 μg/mL		15.2 μg/mL	0.55	[196]
Lamotrigine	2	*– Natural seizure disorder*	–		8.3 mg/L	6.8 mg/L	–/0.82	[197]
		– Fatal intoxication by lamotrigine	54 mg/L		52 mg/L	92 mg/L	1.7/1.8	
Levomepromazine	1	*Drug-related death*		Blood: 0.01 μg/mL		0.53 μg/mL	53	[126]
Lidocaine	1	*Cardiac fibrillation*		Blood: 31 μg/mL		6 μg/mL	0.19	[198]
Lidocaine MGEX[a]	5	*Varied, requiring endotracheal intubation (survival time at hospital 3 to 10 h)*	0.37 μg/mL (0.007–1.18) MGEX: 0.012 μg/mL (ND–0.017)		0.35 μg/mL (0.009–1.35) MGEX: 0.012 μg/mL (ND–0.020)	0.32 μg/mL (0.011–0.69) MGEX:0.11 μg/mL (ND–0.200)	0.86/0.91 MGEX = 9.2/9.2	[199]
Lithium	1	*Mixed-drug intoxication involving tranylcypromine and lithium*	0.57 μmol/L		–	1.42 μmol/L	2.5/–	[200]
Lithium	1	*Fatal intoxication involving lithium*		Blood: 1.93 mequiv./L		15.7 mequiv./L	8.1	[201]
Loxapine	1	*Suicide by acute loxapine overdose*	–		9.5 mg/L	28.8 mg/L	–/3.0	[202]
Maprotiline	1	*Suicide by drug intoxication*	8.6 mg/L		7.3 mg/L	137 mg/L	16/19	[203]
mCPP[a]	1	*Ingestion of mCPP followed by asthma attack that led to death*		NA Embalmment		5.1 ng/mL	–/–	[204]
mCPP[a]	1	*Multiple drug intoxication*		Blood: 325.5 μg/g		945.2 μg/g	2.9	[205]
MDA[a]	1	*Suicide by multiple drug intoxication*	Serum: 0.43 mg/L		–	0.82 mg/L	1.9/–	[206]
MDA[a]	11	*– MDMA intoxication*	–		2.090 μg/mL (0.026–10.083)	9.27 μg/mL (ND–36.447)	–/4.4	[79]
		– Multiple drug intoxication	–		0.484 μg/mL (0.070–1.809)	1.385 μg/mL (0.161–5.522)	–/2.9	
		– Stab wound	–		0.112 μg/mL	0.378 μg/mL	–/3.4	
MDA [a]	1	*Intoxication by MDEA[a] overdose*	Serum: 0.32 mg/L		Serum: 0.34 mg/L	0.44 mg/kg	#/#	[207]
MDDM or MDDA[a]	1	*MDMA[a] overdose*	2.5 ng/mL		21.7 ng/mL	1101 ng/mL	440/51	[208]
MDEA[a]	1	*Suicide by multiple drug intoxication*	Serum: 7.3 mg/L		–	11.3 mg/L	1.5/–	[206]
MDEA[a]	1	*Intoxication by MDEA overdose*	Serum: 12 mg/L		Serum: 22 mg/L	19 mg/kg	#/#	[207]
MDMA[a]	1	*Fatal intoxication involving MDMA*		Blood: 2.9 mg/L		73 mg/L	25	[209]
MDMA[a]	1	*Acute cardiopulmonary failure*	3.1 μg/mL		5.7 μg/mL	14.2 μg/mL	4.6/2.5	[210]
MDMA[a]	2	*– Fatal hyperthermia*	–		0.420 μg/mL	22.075 μg/mL	–/53	[211]
		– Acute cardiopulmonary failure	13.508 μg/mL		–	86.954 μg/mL	6.4/–	
MDMA[a]	*1*	*Overdose fatality involving 4-MTA and MDMA*	10.5 μg/L		16.5 μg/L	231.1 μg/L	22/14	[113]
MDMA[a]	1	*Suicide by multiple drug intoxication*	Serum: 13.3 mg/L		–	23.5 mg/L	1.8/–	[206]
MDMA[a]	11	*– MDMA intoxication*	–		2.453 μg/mL (ND-3.548)	4.467 μg/mL (ND-6.865)	–/1.8	[79]
		– Multiple drug intoxication	–		10.09 μg/mL (1.301–40.41)	31.85 μg/mL (1.262–131.0)	–/3.2	
		– Stab wound	–		1.630 μg/mL	16.02 μg/mL	–/9.8	
MDMA[a]	1	*Intoxication by MDEA overdose*	0.016 mg/L		Serum: 0.02 mg/L	ND	–/–	[207]

Table 2 (*Continued*)

Substance	*n*	Cause of death	Blood concentration		Bile concentration (range)	Ratio Bile/PB & Bile/CB	Ref.
			Peripheral blood	Cardiac blood			
MDPV[a]	1	*Acute death from MDPV*	0.44 μg/mL	0.50 μg/mL	0.88 μg/mL	2.0/1.8	[212]
Mephedrone	1	*Multiple drug intoxication*	Blood: 1.33 mg/L		1.29 mg/L	1.0	[213]
Methamphetamine	1	*Suicide by methamphetamine overdose*	30 mg/L	–	17 mg/L	0.57/–	[129]
Methamphetamine	1	*Ventricular fibrillation/ tachycardia*	Blood: 0.7 mg/L		21.7 mg/L	31	[214]
Methocarbamol	1	*Fatal drug interaction caused by ingestion of methocarbamol and ethanol*	Blood: 257 μg/mL		927 μg/mL	3.6	[215]
Methylone	3	*Suicide by headshot*		0.11 mg/L	0.52 mg/L	–/4.7	[216]
		Drowning	–	0.06 mg/L	0.42 mg/L	–/7.0	
		Cardiac arrest associated with methylone	0.67 mg/l	0.74 mg/L	1.8 mg/L	2.7/2.4	
Metoprolol	1	*Suicide by acute metoprolol overdose*	Blood: 4.7 mg/L		254 mg/L	54	[217]
Metoprolol	1	*Suicide by acute metoprolol overdose*	Blood: 19.8 mg/L		83.1 mg/L	4.2	[218]
Mexiletine	1	*Suicide by acute mexiletine overdose*	14 mg/L	38 mg/L	440 mg/L	31/12	[219]
Midazolam	1	*Fatal intoxication involving fentanyl*	Blood: 7.5 ng/mL		3.3 ng/mL	0.4	[179]
Mirtazapine	7	*Therapeutic use of mirtazapine*	0.17 mg/L (0.04–0.24)	0.19 mg/L (0.03–0.32)	2.76 mg/L (0.40–6.6)	16/15	[220]
Mirtazapine	8	*– Acute ethanol intoxication*	0.07 mg/L	ND	ND	–/–	[221]
		– Acute alcohol intoxication	<0.01 mg/L	<0.01 mg/L	ND	–/–	
		– Narcotic intoxication	0.13 mg/L	0.14 mg/L	0.9 mg/L	6.9/6.4	
		– Cocaine intoxication	0.13 mg/L	0.14 mg/L	1.6 mg/L	12/11	
		– Narcotic and ethanol intoxication	0.05 mg/L	0.05 mg/L	0.42 mg/L	8.4/8.4	
		– Other	0.01 mg/L (ND-0.01)	0.15 mg/L(0.01–0.33)	0.18 mg/L (−0.11 to 0.51)	18/1.2	
Mirtazapine	1	*Adverse effects of mirtazapine initiated a process leading to death*	1030 ng/mL	380 ng/mL	2950 ng/mL	2.9/7.8	[222]
Molidone	1	*Multidrug overdose*	Blood: 6 mg/L		23 mg/L	3.8	[223]
Morphine	1	*Acute heroin fatality*	NA Embalmment		2476 ng/mL	–/–	[152]
Morphine	3	*Not specified*	Blood: trace		7.6 mg/100 g	–/–	[224]
			Blood: trace		9.0 mg/100 g	–/–	
			Blood: 0.4 mg/100 g		14.5 mg/100 g	36	
Morphine	3	*Multiple drug intoxication*	Blood: 140.4 ng/mL (60.5–250.7)		33,018 ng/mL (1211.1–78,906.5)	236	[114]
Morphine	27	*Heroin overdose*	Blood: Free: 0.33 mg/L (<0.05–0.86) Total: 0.61 mg/L (0.1–1.60)		Total: 57.7 mg/L (0.36–330)	Total: 95	[73]
Morphine	1	*Accidental poisoning by morphine*	Blood: 0.128 mg/L		135 mg/L	1054	[225]
Morphine	1	*Heroin overdose*	Blood: ND (death 6 days after intoxication)		21.3 μg/mL	–/–	[84]
Morphine	1	*Possible overdose by heroin*	168 μg/L	–	357 μg/L	2.1/–	[154]
Morphine	1	*Fatal intoxication GHB/Heroin*	0.77 μg/mL		14.3 μg/mL	19	[188]
Morphine	1	*Fatal intoxication involving heroin and ethanol*	Blood: 0.68 μg/mL		0.32 μg/mL	0.47	[226]
Morphine	37	*– Heroin related death With cocaine or metabolite >0.01 mg/L (n = 8)*	Total morphine: 0.33 mg/L (0.12–1.10) Free morphine: 0.23 mg/L (0.04–0.57)		89.5 mg/L (41.0–160)	271	[74]
		– Heroin related death Without cocaine or metabolite <0.01 mg/L (n = 29)	Total morphine: 1.13 mg/L (0.07–5.00) Free morphine: 0.40 mg/L (0.02–1.40)		59.4 mg/L (0.27–259)	53	

Nicotine	1	*Respiratory paralysis*	222 ng/mL	Right chamber: 666 ng/mL Left chamber: 733 ng/mL	1160 ng/mL	5.2/Right: 1.7 Left: 1.6	[227]
Nitrazepam	1	*Nitrazepam overdose and cold exposure*	Right femoral vein: 0.450 μg/mL	Right chamber: 0.973 μg/mL Left chamber: 0.880 μg/mL	4.08 μg/mL	9.0/Right: 4.2 Left: 4.6	[228]
Olanzapine	1	*Hypertensive cardiovascular disease*	–	550 ng/mL	6346 ng/mL	–/12	[229]
Orphenadrine	12	– *Fatal intoxication involving orphenadrine*	Blood: 21.2 μg/mL (5.5–37)		150 μg/mL (85–234)	7.1	[230]
		– *Multiple drug intoxication*	Blood: 14.6 μg/mL (0.04–55)		106.1 μg/mL (0.04–270)	7.3	
Oxycodone	15	– *Multiple drug intoxication*	0.92 mg/L (0.19–2.2)	0.84 mg/L (0.12–1.9)	5.69 mg/L (0.19–23)	6.2/6.8	[77]
		– *Suicide by multiple drug intoxication*	1.19 mg/L (0.98–1.4)	2.35 mg/L (0.75–5.4)	21.7 mg/L (5–49)	18/9.2	
Paclitaxel *(other molecules with taxane ring system were reported)*	1	*Fatal poisoning with Taxus baccata*	<0.5 μg/L	<0.5 μg/L	24 μg/L	–/–	[231]
Paclitaxel *(other molecules with taxane ring system were reported)*	2	*Fatal poisoning with Taxus baccata*	<0.1 μg/L <0.1 μg/L	<0.1 μg/L <0.1 μg/L	24 μg/L 800 μg/L	–/– –/–	[232]
Pancuronium	1	*Fatal intoxication involving pancuronium*	0.7 mg/L	0.7 mg/L	0.4 mg/L	0.57/0.57	[233]
Pentobarbital	1	*Suicide by acute pentobarbital overdose*	13.5 μg/mL	–	67.4 μg/mL	5.0/–	[234]
Pentoxifylline	1	*Acute intoxication involving diltiazem and pentoxifylline and cardiomyopathy*	Blood: 0.63 mg/dL		0.22 mg/dL	0.35	[164]
Perphenazine	1	*Suicide by multiple drug intoxication*	3.5 mg/L	4.4 mg/L	40 mg/L	11/9.1	[145]
Phendimetrazine	1	*Fatal intoxication involving phendimetrazine*	Blood: 0.67 μg/mL		2.03 μg/mL	3.0	[235]
Phenobarbital	1	*Drug-related death*	Blood: 15 μg/mL		36.80 μg/mL	2.5	[126]
Piritramide	1	*Fatal piritramide overdose*	0.1 mg/L	0.32 mg/L	2.51 mg/L	25/7.8	[236]
Platinium (Cisplatin)	1	*Fatal accidental cisplatin overdose*	1213 μg/L	1515 μg/L	501 μg/L	0.41/0.33	[237]
Pregabalin	11	– *Multiple drug intoxication*	22.6 mg/L (5.6–45.3)	27.1 mg/L (4.7–49.4)	61.2 mg/L (12.2–159.4)	2.7/2.3	[238]
		– *Other*	10.5 mg/L (3.8–6.7)	4.2 mg/L (3.8–4.6)	17.6 mg/L (17.0–18.2)	1.7/4.2	
		– *Not reported*	8.78 mg/L (4.4–16.3)	6.6 mg/L (2.7–11.1)	16.9 mg/L (10.5–19.7)	1.9/2.6	
Propofol	1	*Accidental tramadol and propofol intoxication*	–	0.2 μg/mL	0.71 μg/mL	–/3.6	[239]
Propoxyphene	3	*Suicidal poisoning with co-proxamol*	4.6 mg/L	14 mg/L	34 mg/L	7.4/2.4	[118]
			3.2 mg/L	8.7 mg/L	48 mg/L	15/5.5	
			3.9 mg/L	4.2 mg/L	37 mg/L	9.5/8.8	
Quazepam	1	*Ischemic heart disease*	Total: 17.7 ±1.2 ng/mL Free: 18.6 ±1.3 ng/mL	Total: 17.5 ±1.2 ng/mL Free: 19.3 ±0.8 ng/mL	Total: 24.1 ±1.8 ng/mL Free: 27.2 ±1.7 ng/mL	Total: 1.4/1.4 Free: 1.5/1.4	[240]
Quetiapine	4	– *Acute myocardial ischemia due to coronary artery atherosclerosis*	1.0 mg/L	–	6.0 mg/L	6.0/–	[241]
		– *Acute combined ethanol and quetiapine poisoning*	6.0 mg/L	–	33 mg/L	5.5/–	
		– *Suicide by quetiapine overdose*	1.0 mg/L	–	40 mg/L	40/–	
		– *Fatal mixed-drug overdose*	–	11 mg/L	96 g/L	–/8.7	
Quetiapine	3	– *Multiple drug intoxication*	0.95 mg/L	5.1 mg/L	7.5 mg/L	7.9/1.5	[242]
		– *Multiple drug intoxication*	0.26 mg/L	0.43 mg/L	1.3 mg/L	5.0/3.0	
		– *Fatal cocaine intoxication*	<0.10 mg/L	<0.10 mg/L	0.60 mg/L	–/–	
Quetiapine	5	– *Multiple drug intoxication*	Blood: 19.8 mg/L		161.5 mg/L	8.2	[243]
		– *Multiple drug intoxication*	Blood: 2.7 mg/L		46.2 mg/L	17	
		– *Multiple drug intoxication*	Blood: 1.3 mg/L		16.4 mg/L	13	

Table 2 (*Continued*)

Substance	*n*	Cause of death	Blood concentration		Bile concentration (range)	Ratio Bile/PB & Bile/CB	Ref.
			Peripheral blood	Cardiac blood			
		– *Other*		Blood: 0.15 mg/L	10.1 mg/L	67	
		– *Other*		Blood: 2.7 mg/L	16.5 mg/L	6.1	
Rifampicin	1	*Fatal overdose by rifampicin*		Blood: 55 ± 2 μg/mL	313 ± 2 μg/mL	5.7	[244]
Ropinirole	1	*Fatal intoxication involving ropinirole*	64 ng/mL	–	826 ng/mL	13/–	[245]
Sertraline	8	*Civil aviation accident*	Blood: 0.160 μg/mL (0.005–0.392)		3.144 μg/mL (0.187–8.157)	20	[246]
Sertraline	1	*Adverse effects of mirtazapine initiated a process leading to death*	880 ng/mL	–	52,170 ng/mL	59/–	[222]
Sildenafil	1	*Fatal sildenafil-verapamil intoxication*		Blood: 105 ng/mL	1206 ng/mL	12	[247]
Sildenafil	1	*Heart failure due to myocardiosclerosis*		Blood: 0.04 mg/L	0.99 mg/L	25	[248]
Strychnine	1	*Acute strychnine poisoning*		–	2.40 mg/L	–/–	[249]
Strychnine	1	*Suicide by rodenticide poisoning*	0.96 mg/L	0.31 mg/L	1.17 mg/L	12/3.8	[250]
Strychnine	1	*Fatal poisoning of strychnine*	1.82 mg/L	3.32 mg/L	11.4 mg/L	6.3/3.4	[251]
Strychnine	1	*Fatal intoxication involving strychnine*		Blood: 3.3 mg/L	9.2 mg/L	2.8	[252]
Sulindac	1	*Accidental disopyramide and sulindac overdose*		Blood: 12.2 mg/L	1251 mg/L	102	[167]
Tetrahydrophtalimide	1	*Multiple drug intoxication*	0.22 μg/mL	0.35 μg/mL	0.30 μg/mL	1.4/0.86	[253]
THC-COOH[a]	50	*Automobile accident involving marijuana intake*	Blood: 0.081 μg/mL (0.016–0.330)		12.9 μg/mL (1.03–43.7)	159	[80]
Theophylline	1	*Fatal intoxication involving theophylline*		Blood: 250 mg/L	275 mg/L	1.1	[254]
Thiamylal	1	*Hanging*		Blood: 29 mg/L	16 mg/L	0.55	[255]
Thiamylal	1	*Fatal intoxication involving thiamylal*		Blood: 129 mg/L	ND	–	[256]
Thioridazine (TRZ)	1	*Fatal intoxication involving thioridazine*		Blood: enantiomer (+) TRZ = 0.07 mg/L enantiomer (−)TRZ = 0.20 mg/L	enantiomer (+) TRZ = 0.48 mg/L enantiomer (−) TRZ = 1.07 mg/L	enantiomer (+) 6.9 enantiomer (−) 5.4	[257]
Tilidine	1	*Multiple drug intoxication and long exposure at cold temperature*		Blood: 1.74 mg/L	2.88 mg/L	1.7	[258]
Tioridazine	1	*Drug-related death*		Blood: 2.06 μg/mL	1.62 μg/mL	0.79	[126]
Tizanidine	1	*Suicide by multiple drug intoxication*	–	2.34 mg/L	3.37 mg/L	–/1.4	[259]
Topiramate	1	*Seizure disorder with upper respiratory infection*		Blood: 8.9 mg/L	10.9 mg/L	1.2	[260]
Topiramate	1	*Fatal intoxication involving topiramate*		Blood: 49 mg/L	48 mg/L	0.98	[141]
Tramadol	1	*Severe depression of fundamental function as consequence of acute intoxication of tramadol*	61.83 μg/mL	–	107.94 μg/mL	1.7/–	[261]
Tramadol	1	*Accidental intoxication with tramadol and propofol*	–	5.3 μg/mL	15 μg/mL	–/2.8	[239]
Tramadol	1	*Fatal tramadol intoxication*	9.6 mg/L	13.1 mg/L	46.1 mg/L	4.8/3.5	[262]
Tranylcypromine	1	*Mixed-drug intoxication involving tranylcypromine and lithium*	0.19 μg/mL	–	0.35 μg/mL	1.8/–	[200]
Triazolam	1	*Postural asphyxia caused by triazolam poisoning*	Right femoral vein: 62 ng/mL	Right chamber: 153 ng/mL Left chamber: 90 ng/mL	1130 ng/mL	18/Right: 7.4 Left: 12.6	[263]

Trimetazidine	1	*Fatal sildenafil-verapamil intoxication*		Blood: 2133 ng/mL	553 ng/mL	0.26	[247]
Valproic acid	1	*Fatal intoxication involving valproic acid*		Blood: 1050 mg/mL	713 mg/mL	0.68	[264]
Vardenafil	1	*Fatal aviation accident*	–	291 ng/mL	1665 ng/mL	–/5.7	[265]
Venlafaxine	9	*– Suicide by venlafaxine overdose (combined drug toxicity)*	7.2 mg/L	–	10.6 mg/L	1.5/–	[266]
		– Suicide by venlafaxine overdose (combined drug toxicity)	31 mg/L	–	46 mg/L	1.5/–	
		– Suicide by venlafaxine overdose (combined drug toxicity)	36 mg/L	–	53 mg/L	1.5/–	
		– Other	1.7 mg/L (0.1–6.6)	–	5.02 mg/L (<0.05–20)	3.0/–	
Venlafaxine	1	*Suicide by mixed-drug intoxication*	6.2 mg/L	–	54 mg/L	8.7/–	[267]
Venlafaxine	2	*– Suicide by acute venlafaxine overdose*	–	6.4 mg/L	100 mg/L	–/16	[268]
			–	84 mg/L	290 mg/L	–/3.5	
Verapamil	1	*Overdose by verapamil*		Blood: 16.5 mg/L	62.6 mg/L	3.8	[269]
Verapamil	1	*Fatal sildenafil-verapamil combination*		Blood: 659 ng/mL	3129 ng/mL	4.7	[247]
Xylazine	1	*Hanging*	6.3 mg/L	2.9 mg/L	0.01 mg/L	0.02/0.03	[270]
Zaleplon	3	*– Suicide by multiple drug intoxication*	<3.0 ng/mL	13 ng/mL	<3.0 ng/mL	–/–	[271]
		– Pulmonary thromboembolism	–	55 ng/mL	85 ng/mL	–/1.5	
		– Drowning/Effect of multiple drug intoxication	503 ng/mL	227 ng/mL	33 ng/mL	0.07/0.15	
Zaleplon	1	*Multiple drug intoxication*	–	2.2 mg/L	8.6 mg/L	–/3.9	[272]
Zimelidine	1	*Suicide (gunshot)*		Blood: 0.71 mg/L	Free: 1.4 mg/L Total: 3.8 mg/L	Free: 2.0 Total: 5.4	[273]
Zipeprol	1	*Fatal overdose involving zipeprol*	–	6.69 mg/L	55.56 mg/L	–/8.3	[274]
Zolpidem	2	*Suicide by zolpidem overdose*	Blood (subclavian) = 4.5 mg/L; (iliac) = 7.7 mg/L		8.9 mg/L	Subclavian:2.0 Iliac:1.2	[275]
				Blood (iliac) = 1.6 mg/L	2.6 mg/L	1.6	
Zolpidem	1	*Suicide by zolpidem overdose*		Blood: 3.29 mg/L	1.27 mg/L	0.39	[116]
Zopiclone	1	*Suicide by zopiclone overdose*	254 ng/mL	408 ng/mL	114,700 ng/mL	451/281	[276]
Zopiclone	1	*Fatal intoxication involving zopiclone*	Left femoral vein: 1.2 μg/mL	Superior vena cava: 1.4 μg/mL	14.1 μg/mL	12/10	[277]

ND, not detected; NQ, detected but Not Quantified; # ratio not calculated because units of blood and bile values are not the same.

[a] 4-MTA, 4-methylthioamphetamine; 6MAM, 6-monoacetyl-moprhine; AH-7921, 3,4-dichloro-N-[(1-dimethylamino)cyclohexylmethyl]benzamide; BZE, benzoylecgonine; EME, ecgonine-methylester, DMT, dimethyltryptamine; GHB, gamma-hydrxybutyric acid; mCPP, meta-Chlorophenylpiperazine; MDA, 3,4-methylendioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDDM or MDDA, 3,4-methylenedioxy-N,N-dimethylamphetamine; MDPV, methylenedioxypyrovalerone; MGEX, monoethylglycinexy-lidide; THC-COOH, 11-nor-9-carboxy-delta9-tetrahydrocannabinol.

These differences between blood and bile results showed a longer detection window in bile, making it interesting for the detection of short half-life molecules in cases with long intake-to-death interval, as may be the case with heroin [83,84] (6-monoacetyl-morphine (6MAM) half-life = 6–25 min, morphine half-life = 2–3 h [85]) and colchicine (half-life = 20 min) [86,87]. Thus, as several authors claim [16,17,78,80], bile may be the matrix of choice for drug of abuse screening when blood concentrations are low or non-detectable (weak exposure or long intake-to-death interval) or blood is not available.

Bile has also been recommended as a complementary matrix to blood for general unknown screening. In an autopsy series ($n = 5$), Moriya et al. [88] highlighted the qualitative interest of bile, as concentrations of 8 molecules were systematically higher than in blood (mean bile/blood ratio, 5.74). In a series of 44 autopsies, Vanbinst et al. [17] compared bile and blood concentrations for 26 medicines or their metabolites: bile/cranial blood ratios ranged from 1,08 (paracetamol) to 1.944 (desclobazam). A brief review of the literature entitled "Significance of bile analysis in drug-induced death" [16] cited several case reports to show that routine bile analysis would be useful, as concentrations for most molecules are significantly higher than in blood; even when no molecules are detected in blood, toxicology results should not be considered negative without complementary bile analysis. As bile contains a large proportion of metabolites, as in the case of cocaine, nitrobenzodiazepines or THC [57,64,89], it is useful to include metabolites in the screening.

However, molecules detected in blood may in some cases go undetected in bile. In the study by Vanbinst et al. [17], in 6 cases out of 44 (13.6%), molecules found in blood (clobazam, cocaine, codeine, 3,4-methylendioxyamphetamine (MDA), northiaden, 7-acetamidoflunitrazepam) were not detected in bile. Many hypotheses were put forward but not discussed, one of the most interesting being rapid death. The analytic method may be more sensitive in blood than in bile. In one study, meprobamate was quantified in blood but not bile in 13 cases out of 124 [90]: quantification limits for blood and bile were respectively 2.0 and 5.9 μg/mL.

4.3. Quantitative interpretation

4.3.1. Medical drugs and drugs of abuse

Quantitative interpretation of bile concentrations has been rarely reported. Fernandez et al. [57] found poor correlation ($r = 0.48$) between blood and bile concentrations of cocaine derivatives in 20 cases, with bile/blood ratio ranging from 2.15 to 107.53. One limitation of their study was the use of a radioimmunoassay (RIA) which failed to distinguish between cocaine and its metabolites. In another study [88], of 5 autopsy cases, there was no significant correlation between bile and blood concentrations for 8 substances taken together (diazepam, atropine, lidocaine, ephedrine, norephedrine, methamphetamine, amitriptyline, nortriptyline). The limitation of this study was the small number of cases ($n = 5$), obliging the authors to combine the 8 neutral or alkaline molecules despite the fact that they showed widely different bile/blood ratios (between 1.83 and 23.5).

A 19-case series compared blood and bile concentrations of nitrobenzodiazepines (nitrazepam, flunitrazepam, clonazepam) and their 7-amino metabolites [89]. Correlation coefficients were 0.695 for parent molecules and 0.829 for metabolites. The study also included other alternative matrices (vitreous humor, liver and urine), and concluded that the correlation was "encouraging" for improving quantitative interpretation of nitrobenzodiazepine-positive cases by analyzing several matrices postmortem. In a series of 124 autopsies, meprobamate was analyzed simultaneously in blood and bile and a number of statistical models were applied to help in interpreting the bile concentrations [90]. A significant correlation emerged ($r = 0.66$) despite a certain scatter when blood concentration was higher than 100 mg/L. Secondly, two groups were distinguished according to therapeutic (<50 mg/L) versus supratherapeutic blood levels (>50 mg/L). A bile concentration threshold of 53 mg/L was statistically equivalent to the blood concentration threshold of 50 mg/L, with 0.95 sensitivity and 0.93 specificity in this population.

Bevalot et al. [91] combined an animal experiment with a series of autopsy cases on 6 test substances (meprobamate, morphine, cyamemazine, caffeine, diazepam, and citalopram) to assess both qualitatively and quantitatively the use of bile as alternative matrix to blood. The concentrations of these 6 substances were determined by GC/MS-MS in rabbit specimens collected at various delays between injection and euthanasia and in human postmortem samples. Qualitative interest of bile analysis was indisputable as all molecules detected in blood were found in bile. Moreover animal experiment demonstrated a wider time detection window for bile than blood. Quantitative interpretation was limited for molecules with the highest bile/blood concentrations ratios (morphine, citalopram, cyamemazine) as a poor correlation was observed. Diazepam showed excellent correlation in bile specimens from rabbits ($r = 0.961$, $n = 20$) but a poor correlation in human postmortem cases ($r = 0.447$, $n = 23$). Remarkably, for molecules with low bile/blood concentrations ratios (closed to 1): cyamemazine and meprobamate, correlation between these two matrices were significant. However, due to non-negligible scatter, the authors advised to infer blood concentrations from bile ones with caution or using a statistical approaches which allows estimation of uncertainty.

4.3.2. Gamma-hydroxybutyric acid

Due to the difficulty of interpreting postmortem blood concentrations of gamma-hydroxybutyric acid (GHB), complementary alternative matrices have been used to distinguish endogenous origin or postmortem neoformation from exogenous input [92]. The two studies published concerning bile in this context reported divergent results. Moriya [93] selected cases in which inquest data ruled out exogenous input of GHB or gamma-butyrolactone (GBL) and in which the postmortem interval was not greater than 48 h (in order to limit the likelihood of postmortem neoformation). For the 9 cases studied, bile concentrations were homogeneous and low (from <LOD (0.5 μg/mL) to 2.8 μg/mL). The author considered bile to be an alternative matrix of choice as endogenous GHB levels are low and suggested a 10 mg/L bile threshold to distinguish endogenous versus exogenous GHB. Kintz et al. [94] examined 14 cases in which exogenous exposure to GHB was unlikely but where cardiac blood concentrations were above the 50 mg/L threshold consistent with endogenous formation. For these 14 cases, femoral blood and vitreous humor concentrations were below the 50 mg/L threshold, whereas bile concentrations displayed wide scatter (6.1–238 mg/L, $n = 7$). In this second study, the authors concluded that bile did not meet the prerequisites for interpreting GHB concentrations.

4.3.3. Ethanol

Many studies have focused on interpreting ethanol concentrations in alternative matrices when no blood is available or to confirm a high blood concentration thought to be due to postmortem redistribution or neoformation. Kugelberg et al.'s review [95] of the interpretation of ethanol analyses merely mentions bile as being one of the non-conventional samples. Nevertheless, it was the focus of numerous reports, especially in the 1980s and early 1990s. Thus, bile ethanol concentration correlated strongly with blood ethanol ($r = 0.927$, $n = 37$ [96]; $r = 0.9107$, $n = 189$ [97]; $r = 0.94$, $n = 100$ [98]); mean bile/blood

Table 3
Bile/blood ethanol concentration ratios in the literature.

Ref.	Number of cases	Bile/blood ethanol concentration ratio		
		Mean	SD	Range
[99]	78 [ethanol] = 0.02 à 0.49%	0.92	0.22	–
[96]	37	1.00	0.19	0.45–1.45
[97]	189	1.03	0.29	0.32–2.91
[97]	129 Bile concentration weighted for lipid content	1.00	0.29	0.40–2.58
[101]	5	–	–	0.7–1.12
[98]	100	1.1	0.3	0.8–1.4
Cited in [42]	–	–	–	0.7–1.4

ratios were close to 1 (Table 3). Even so, extrapolating blood concentrations from bile requires caution: the studies as a whole showed wide scatter for bile concentrations as seen from the wide range of bile/blood ratios (Table 3). Stone and Rooney [99] reported greater ratio variation for ethanol concentrations less than 0.1% (CV = 34%) compared to concentrations higher than 0.1% (CV 14%; data corrected in a letter to the editor [100]). Winek et al. [97] showed that scatter was reduced if bile ethanol concentration was corrected for sample lipid content. Intake-to-death interval may also account for the scatter: Christopoulos et al. [101], in an 8-case series, reported bile concentrations to be higher than in blood in deaths rapidly following massive alcohol intake (<15 min, $n = 3$) and lower in slower death. Taken together, these findings show that, despite significant correlation between the two matrices, blood concentration is hard to extrapolate from bile data alone. Winek et al. [97] described an approach taking account of this uncertainty, reporting bile extrapolation as estimated blood concentration ranges with associated uncertainty values.

In plane accidents or in explosions in which body damage is often extreme, negative findings in bile or other matrices (urine, brain, kidney, etc.) help in demonstrating postmortem ethanol neoformation suspected in certain positive blood samples [102,103]. On the other hand, in a study of ethanol analysis in decomposed cadavers [104], postmortem neoformation was suspected in 55 cases: ethanol was detected in blood and in bile whereas ethanol levels in urine and vitreous humor remained low or equal to zero. Thus, as in any biological sample, bacterial contamination may induce ethanol production in bile. Bile, when available, can be used to screen for ethanol to distinguish neoformation from antemortem consumption of alcohol, associated to analysis of other biological fluids and study of circumstances of death. Screening for ethylglucuronide, a minor ethanol metabolite, is often useful in this problematic: one study showed ethylglucuronide to be strongly excreted into bile, with concentrations higher than in blood [105].

4.4. Postmortem redistribution

Gallbladder bile has been studied both as a possible source of postmortem redistribution due to high xenobiotic content and as a target for molecule diffusion from anatomically close abdominal organs.

Postmortem redistribution was studied in rabbit according to euthanasia-to-sampling interval following intravenous administration of 3 beta-blockers ($n = 18$, interval: 0–48 h) [106] and of MDMA ($n = 15$; interval: 0–72 h) [107]. Both studies focused on bile concentration evolution, finding reduction with postmortem interval. In both studies, bile concentrations were higher than in the liver and xenobiotic diffusion from bile to liver may have contributed to the postmortem increase in liver concentration. For beta-blockers, the reduction in bile concentration may have contributed to a concomitant non-significant increase in peripheral blood concentration in the inferior vena cava. Finally, bile release into the duodenum and then in the stomach could partially account for early detection of beta-blockers in the stomach despite administration having been intravenous according to the authors. This hypothesis of duodenal-gastric reflux of bile was put forward in a study focusing on gastric morphine concentrations in intravenous heroin overdose [73]. Another study focused on postmortem redistribution of THC in pig ($n = 15$) [108] after intrajugular administration, with postmortem intervals of 0–48 h; bile and liver concentrations seemed not to vary with postmortem interval, but significant findings were prevented by the low concentrations found, which were the lowest of all the tissues studied (bile, 1.4–7.5 ng/g; right liver, 2.8–8.2 ng/g; left liver, 4.0–8.0 ng/g).

The stomach has been studied as a possible source of postmortem redistribution. It may constitute a reservoir of xenobiotics if absorption was incomplete at time of death. De Letter et al. [109] simulated this by postmortem infusion of MDMA into the stomach or trachea (to simulate gastro-esophageal reflux) in rabbit. Pounder et al. studied the hypothesis by postmortem instillation of a medical drug cocktail (amitriptyline, paracetamol, lithium) in human cadaver [110] and 8 cases of medical drug overdose. Taken together, these results showed diffusion from the stomach toward the left liver, generally sparing the right lobe. Only Pounder et al. [110] focused on bile concentrations, reporting diffusion to the gallbladder, although less than to the liver.

Finally, few significant studies of postmortem redistribution have focused on bile. These studies, cited above, disclosed a tendency for levels to diminish over time due to xenobiotic diffusion toward neighboring tissue with lower concentrations and a mild contamination of the gallbladder by the stomach in case of massive storage of xenobiotics in the stomach contents during the absorption phase.

5. Conclusion

Postmortem forensic toxicological investigation aims to assess xenobiotic intake and its relation to cause of death. Compound detection in a biological matrix enables the first, qualitative, level of interpretation. Interpreting concentrations in terms of effect (therapeutic, toxic, impaired vigilance, etc.) is the quantitative aspect, and generally based on blood concentration interpretation. The present review of the literature sought to update the field of knowledge as it can be applied to the use of the bile matrix in forensic toxicology. Gallbladder bile is an additional autopsic sample for analysis alongside other matrices (blood, stomach contents, etc.) or when no analyzable blood sample is available.

Postmortem bile sampling is easy to perform at autopsy; the essential factors to consider may be the fullness and postmortem evolution of the gallbladder. Data are sparse on bile storage and xenobiotic stability in bile, and the precautions recommended for any biological sample are applicable with respect to use of preservative agents and storage temperature. Certain authors have suggested that bile, due to its composition (bile salts, etc.) and

alkaline pH, is not a favorable medium for bacteria, although bacterial neoformation of ethanol in bile have been reported. Many sample pretreatment and analytic techniques have been described for bile analysis. The specific composition of bile may lead to interferences and matrix effects, and it is important to bear these in mind in developing an analytic method in order to ensure the reliability of detection and measurement.

Biliary excretion is a complex process. It should be stressed that knowledge of the biliary route is poorer than for other matrices such as urine or blood, especially for compounds of forensic interest, as in vivo access is obviously limited. Biliary excretion appears to be strongly molecule-dependent, particularly with respect to molecular weight, lipophilicity and affinity toward hepatocyte membrane transporters, and is based on active processes. This general knowledge, added to a large number of case reports and a few autopsy series, suggests that bile concentrations are often greater than in blood and that the concentration ratio may be very high. Bile has therefore been suggested as matrix of choice for screening of medicines and of drugs of abuse. The detection window for molecules significantly excreted in bile is longer than in blood if an equally effective analytic method is performed, notably in terms of detection limits. This may be of special interest for molecules with short half-life when the intake-to-death interval is long (hospitalization, coma, cranial trauma, etc.), as widely reported in the literature for opiates and colchicine. Despite these qualitative advantages, bile is generally used for screening only when urine, which is an elimination medium easier to analyze, is absent.

There has been little research on bile for the quantitative interpretation of xenobiotic concentrations. Even so, significant correlation has been demonstrated between bile and blood concentrations for certain xenobiotics. Thus quantitative interpretation of bile data is conceivable in the absence of any analyzable blood. However, the wide scatter of concentrations in bile, as in many other alternative matrices, requires tools to be developed taking this uncertainty into account. Determining a bile threshold for overdose [90] or a concentration range corresponding with a known probability to the concentration measured in bile [97] have been suggested. Finally, interpretation should also take account of possible postmortem redistribution.

In conclusion, the present review of the literature highlights not only the qualitative interest of bile but also a possible quantitative interest in the case of several molecules. There are gaps in our knowledge of bile excretion, and a lack of robust studies of the interest, limitations and implementation of bile analysis in forensic toxicology. Excretion mechanisms play a determining role in the interpretation of bile analysis results. From the qualitative point of view, it would be very useful to know, or even predict, which molecules of forensic interest are strongly secreted, and thus easily detected, in bile. Better knowledge of hepatocytic active transport and its substrates is needed. From the quantitative point of view, correlations between blood and bile concentrations have been demonstrated for several molecules. These seem to show certain common features: ethanol, meprobamate, cyamemazine and, to a lesser degree, nitrobenzodiazepines, are all small molecules with low bile/blood concentration ratios. It would thus be interesting to describe the properties of molecules and the biliary excretion or reabsorption mechanisms underlying these correlations.

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