The Utility of Bile in Postmortem Forensic Toxicology

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ABSTRACT

Bile is one matrix type that may be collected at autopsy and submitted to the toxicology laboratory for analysis. Because it is an excretion product of the liver, it can be used for screening purposes and to determine what drugs an individual used or was exposed to prior to death. This paper presents collection and analytical considerations of bile, and provides an overview of its utility from a testing and interpretation perspective. *Acad Forensic Pathol.* 2018 8(2): 324-327

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INTRODUCTION

Toxicology laboratories that perform postmortem testing must be able to process and handle matrices beyond the traditional blood and urine. Ideally, sufficient and varied biological material should be collected from the deceased individual; however, this is not always possible. Specimen source and quality impacts the ability of laboratories to provide the information required by the forensic pathologist or other death investigators. From an interpretation perspective, the challenge is that different matrix types have different utilities; one is not a complete substitute for another. For example, blood is used to determine if a drug was present at a therapeutic, toxic, or potentially lethal concentration at the time of death while positive findings in urine demonstrate past drug use and/or exposure. Forensic practitioners have no control over the condition of a body, or the choice and quality of the specimens that are available for removal and collection. When there is no blood or limited blood volume available, alternate matrix types need to be tested.

DISCUSSION

Bile in Forensic Toxicology

One alternative specimen that may be considered is bile. Bile is a body fluid that aids in the digestion of lipids. It is continuously produced by the liver, concentrated and stored in the gallbladder, and subsequently released into the duodenum. The consistency of bile varies among individuals, but it is typically has a yellow-greenish pigment with a slightly alkaline pH (1, 2). Some reasons for choosing bile as an alternative matrix include ease of collection, large sample volume, extended detection window relative to blood, and high concentrations of drugs and metabolites. Collection of bile is straightforward. It can be performed by syringe aspiration from the gallbladder or the more viscous samples can be obtained by excising the gallbladder and emptying its contents into a container of suitable size. A large volume of bile, approximately 50 mL, may be obtained from the gallbladder if the entire contents are collected. Like other toxicology specimens, if ethanol is an analyte of in-

terest, the sample should be mixed with 1% sodium fluoride to mitigate its neoformation (3).

Drugs and their metabolites are excreted by the liver into the bile, which is then stored and concentrated in the gallbladder. Once in the gallbladder, drugs may undergo a process known as enterohepatic circulation where drugs and/or metabolites are secreted from the bile into the intestine. Once in the intestine, drugs are available to be reabsorbed by the liver to either enter systemic circulation through the hepatic vein or be secreted back into the bile thus, increasing the concentration of drugs present in bile and the amount of time these drugs may be detected (4). While the processes that govern which drugs are excreted into the bile are complex and not completely understood, most abused drugs and many prescription medications have been detected in bile. Abused drugs such as cocaine, heroin, fentanyl, gamma hydroxybutyrate (GHB), ketamine, 3,4-methylenedioxymphetamine (MDA), 3,4-methylenedioxymphetamine (MDMA), methamphetamine, and marijuana in addition to medications such as amitriptyline, buprenorphine, duloxetine, nizatrazepam, phenobarbital, and zolpidem have been confirmed and quantitated in bile from forensic cases (2, 4, 5).

Pharmacokinetic studies that serve to demonstrate expected concentrations, either peak (C_{max}) or steady-state, by dosage and route of administration are most often conducted in blood, serum, or plasma specimens. As a result, quantitative confirmation testing most often takes place in the blood matrix or samples (e.g., serum or plasma) collected at the time of hospital admission. This permits the comparison of analytical results to reference ranges established from drug time course studies. The utility of bile in forensic toxicology is limited due to variable bile-to-blood concentration ratios and limited published reference data. For these reasons, bile is more useful as a qualitative screening matrix similar to urine or vitreous fluid rather than a quantitative confirmatory matrix such as blood.

Testing

Laboratories have designed analytical approaches to accommodate the testing of bile. Even though it is a biological fluid, like blood and urine, it has long been recognized in the toxicology community that from an analytical perspective, a method shown to be suitable by proficiency testing for one matrix does not translate into suitability for another matrix. During the validation phase, method parameters such as accuracy, specificity, sensitivity, and recovery should be evaluated, and in accordance with requirements promulgated by those regulatory agencies that develop consensus standards and guidelines to ensure a sufficient scientific basis exists for work performed (6). A reality in the postmortem arena is that specimens may arrive for testing in a variety of non-ideal conditions including discolored, dried, viscous, and/or decomposed. In this regard, the laboratory must ensure that the method is robust in its ability to handle the non-ideal specimen. A review of the literature shows that several analytical options exist for preparing a bile sample for instrumental analysis including liquid-liquid extraction (LLE) and solid-phase extraction (SPE) (2, 7, 8). While it is possible to apply methods designed for the detection of drugs in blood directly to bile specimens, due to the potential for interference from drug metabolites concentrated in the specimen, additional sample purification is recommended to ensure appropriate identification of drugs.

Confirmations may be qualitative or quantitative, depending upon the intended use of results. In either circumstance, the use of analyte-specific labeled internal standards will best compensate for matrix variations from sample to sample. Another option, in the absence of commercial availability and to circumvent the cost associated with a custom synthesis, is to employ the technique of standard addition (6). Calibration performed using bile that has tested negative for the presence of the drug of interest can be used, but with the variety of specimens that often require analysis the approach of matrix-matched calibration standards is not practical and, by itself, still may not compensate for specimen-specific differences.

Another aspect of testing that, at least for some drugs, may influence the ability of the method to detect certain analytes is if the bile undergoes a hydrolysis step prior to analysis. Some drugs, via phase II metabolism, undergo conjugation to facilitate elimination. A classic example is seen with the opiate drug class. Opiates such as morphine, hydromorphone, and oxycodone undergo extensive glucuronide conjugation. The worst case scenario is that the majority of drug is present in conjugated form(s) and is not detected because the analytical method only detects free or unconjugated drug. Acidic or enzymatic hydrolysis is often used to break this bond. Traditional hydrolysis methods have been used successfully in bile specimens (2, 6). Depending upon the sensitivity of the analytical technique, this may or may not be necessary.

Interpretation

Finally, interpretation of bile results is rather straightforward. Results go to demonstrate past use and/or exposure to a drug, and due to enterohepatic circulation may persist in bile for a longer time period as compared to blood, as previously described (2, 3). Another critical point is that bile drug concentrations can be influenced by water content and postmortem changes. As a consequence, quantitative concentrations in bile have limited direct correlation to circulating blood-drug concentrations (2, 9–12). Even though bile-to-blood drug ratios have been reported, the use of a bile concentration to mathematically calculate a blood concentration would be a risky endeavor. While it could be assumed that bile drug concentrations that appear elevated are indicative of an elevated blood concentration, this cannot be determined without analytical testing of the blood. Drug distribution studies, where drug and drug metabolite concentrations are determined in different bodily fluids including bile, may help to substantiate the cause of death determination.

CONCLUSION

Overall, the bile matrix is best utilized for screening purposes when limited blood sample is available. Under this circumstance, the bile should be used as

a qualitative indicator of what drugs are present in blood. Based upon factors such as the case history, autopsy findings, and the bile-drug concentrations relative to each other, selective quantitative confirmation testing can be performed in blood or an alternative matrix such as vitreous fluid.

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