Journal of Forensic and Legal Medicine 23 (2014) 37–43

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Case report

Determination of cocaine and its major metabolite benzoylecgonine in several matrices obtained from deceased individuals with presumed drug consumption prior to death

Eduardo Alvear, Chemical Pharmacist [a], Dietrich von Baer, PhD [b,*], Claudia Mardones, PhD [b], Antonieta Hitschfeld, Biochemist [b]

[a] Servicio Médico Legal de Concepción, Laboratorio, Camino a Penco 4018, Concepción, Chile
[b] Universidad de Concepción, Facultad de Farmacia – Depto de Análisis Instrumental, Barrio Universitario s/n, Concepción, Chile

ARTICLE INFO

Article history:
Received 17 May 2013
Received in revised form
5 November 2013
Accepted 19 January 2014
Available online 28 January 2014

Keywords:
Cocaine
Benzoylecgonine
Alternative matrices
GC–MS
Postmortem distribution

ABSTRACT

In the field of forensic toxicology, femoral blood is the most useful sample for the determination and quantification of drugs; however, cases in which blood is unavailable are common. In such cases, validated methodologies for drug determination in alternative matrices can be decisive in the investigation of a case. In particular, when femoral blood is unavailable for analysis for the presence of systemic exposure to cocaine and its principal metabolite, benzoylecgonine, validated methodologies from matrices other than blood that can be obtained in the autopsy room would be useful to the forensic toxicologist in the evaluation of a specific forensic case. To address this issue, we implemented and compared in our study the systematic evaluation of extraction, chromatographic separation, and quantification of cocaine and benzoylecgonine in different biological matrices (right and left cardiac blood, femoral arterial and venous blood, urine, vitreous humor, cerebrospinal fluid, brain accumbens nucleus, brain ventral tegmental area, and liver). The studied matrices were those most likely to be obtained from different autopsy rooms at the time of forensic testing in deceased individuals who are presumed of antemortem drug consumption. Solid phase extraction of analytes from the different matrices was performed using C-8/SCX mixed-phase columns, and gas chromatographic mass spectrometry separation was performed using detection in single-ion monitoring mode. The methodological validation was performed for all the studied matrices, and the results showed similar sensitivity and recoveries without statistical differences between the studied matrices. The methods were applied to evaluate a thanatological case using all the study matrices, showing unequal postmortem distribution of cocaine and benzoylecgonine throughout the different matrices tested. The present work opens the option of applying appropriate methodologies in the analysis of matrices, other than the usual blood, to obtain reliable results that may help clarify a forensic case. In addition, we present findings from different studies. This work affirms not only the potentiality of obtaining reliable data but also reaffirms the challenge of applying these data and taking into account the complexity of interpreting results in matrices other than blood.

1. Introduction

Cocaine is one of the most powerful known natural stimulants of the central nervous system and is also highly addictive. The prevalence of drug consumption in Chile has decreased from 2008 to 2010. according to the Ninth National Drug Study in the General Population report in 2011 by the National Service for Prevention and Rehabilitation of Drug and Alcohol Consumption.[1] This study showed that compared with more expensive drugs, marijuana is the most commonly consumed drug in Chile. However, the geography and boundary conditions of Northern Chile, together with the availability and lower price of cocaine in this country, have recently led to the potential consuming population among the residents in the northern zone to rapidly becoming an important percentage. This has been reflected in toxicological results obtained from multiple autopsies performed by regional forensic services.

In 2008, Iquique Forensic Service, located in a northern zone of the country and bordering the countries of Peru and Bolivia,

* Corresponding author.
E-mail address: dvonbaer@udec.cl (D. von Baer).

1752-928X/$ – see front matter © 2014 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.
http://dx.doi.org/10.1016/j.jflm.2014.01.003

performed 200 autopsies corresponding to forensic cases, from which 131 were derived to the toxicological laboratory. Of these cases, 27 (20.6%) tested positive for cocaine in the blood, according to the toxicological examination performed. Nevertheless, according to the characteristics of the forensic case or the circumstances that led to the death of the person (degree of destruction of the body of the deceased), in some occasions, it was not possible to rely on a femoral blood sample, which is the internationally recommended matrix for the testing of cocaine and its products.[2] Another factor that may affect postmortem studies investigating cocaine is the amount of time that has elapsed between death and sampling. This is of particular importance, as postmortem redistribution and degradation make it impossible to determine the blood concentration of cocaine to help explain the cause of death, even with certain presumption of prior use.

Given the importance of identifying the presence of cocaine and its major metabolite, benzoylecgonine (BZE), in cases of the forensic deceased, validation parameters are necessary for the accurate determination and quantification of cocaine and BZE in different matrices that could be sent from the autopsy room to the toxicological laboratory. Previous literature has described methodologies for the quantification and identification of cocaine in some matrices other than blood.[3–13] These methods could be used in the event that femoral blood is unavailable. However, most publications investigate the matrices individually and do not identify a wider range of alternate options. Nevertheless, some publications suggest methodologies for identifying cocaine and its metabolites in several liquid matrices such as blood, plasma, urine, and oral fluid, which we used as the initial support for the development of this work.[8,10–17] These methods have broadened the range of possibilities available to both forensic toxicologists and coroners, especially when faced with a complicated autopsy in the context of bodily integrity, in terms of the analysis of the samples that potentially available in a routine autopsy procedure.[12–24]

In forensic toxicology, it has been proposed that cadaver blood samples be taken directly from the femoral vein after being linked to the inguinal zone to avoid contamination from the abdomen. Due to its peripheral condition, this vein is relatively less affected by postmortem redistribution.[2] The use of vitreous humor (VH) has also been recommended because of its scarce irrigation and the protection that the bony skull structure provides to the ocular globe, which preserves the concentration of xenobiotics, and is very close to the systemic blood circulation.[2,25] Commonly abused drugs, particularly cocaine, have been detected in VH.[26] Furthermore, in an autopsy, a greater diversity of specimens can be collected, including hair, muscle, brain, fat, and lung.[3,8,12,13,27] It is important to note that the matrix chosen for analysis is frequently dictated by the case under study and/or the condition of the deceased body. Nevertheless, the most common specimens for cocaine analyses in postmortem cases are blood, liver, and urine.[5,8,10.11,14,15,17]

In cases of extreme putrefaction, muscle tissue, hair, and bone may be used as appropriate specimens; nevertheless, the physical state of the deceased body will determine which specimens are possible to collect.[27] Liver has always been a key tissue in postmortem toxicological analyses, and the result obtained from this tissue is frequently complementary to any other result obtained from blood. It must be taken into account that the diffusion of cocaine is possible from the large intestine; therefore, using deep tissue from inside the right lobe of the liver is preferred.[28] Brain tissue has also been used for years by toxicologists, mainly in determining the concentration of a substance in a tissue where many toxic substances exert their effects. However, given the unequal distribution of the drugs and the frequent location of action sites in the brain for the different abused drugs, accurate interpretation is more difficult than in peripheral tissues.[29] However, the National Institute on Drug Abuse indicates that at the brain level, one of the neuronal systems that appear to be more affected by cocaine, the ventral tegmental area (VTA), originates in a very deep region of the brain.[30] Nerve cells that originate in the VTA extend into a region of the brain known as accumbens nucleus (AN). Furthermore, this report suggests that during a gratifying event, VTA neurons considerably increase the amount of dopamine released into the AN. During the normal communication process, a neuron releases dopamine inside the synaptic space. Dopamine is linked to its specific receptors in the adjacent neuron, sending a message to it. Cocaine interferes with this normal communication process mediated by neurotransmitters.[30.35]

Currently, many published works use similar analytical procedures for cocaine assay. In operations prior to analysis, solid-phase extraction (SPE) is a widely accepted technique, which is applied to the extraction of different abuse drugs, including cocaine and its metabolites, from different matrices.[8,11] Solid-phase microextraction (SPME) is also used[15,31] with good results, but on a smaller scale than SPE owing to the higher initial cost of the inputs required and because it is not manually applicable to a great number of samples simultaneously.

The main aim of this research was the systematic comparison of cocaine and BZE determination in different matrices, considering the analytical advantages and limitations from the point of view of their application in the study of postmortem distribution of these drugs.

2. Case report

This work is mainly focused on validating methodologies for both the determination and quantification of cocaine and the principal metabolite BZE in matrices other than femoral blood. We also investigated a case where it was possible to include all assessed matrices. In this sense and as an example, the forensic case Prot 141 was considered. This case investigated a 22-year-old man who was 1.72 m in height, with a body mass index of 23.66. The autopsy and sampling data were performed 11.5 h after death. The precise circumstance of the death was asphyxiation by hanging (suicide), without apparent participation of third parties.

During the autopsy, the pathologist collected femoral venous blood, according to routine, and all of the other matrices were collected as part of this study. The final report sent from the forensic laboratory showed only the femoral venous blood results. Nevertheless, the differences in concentration in other matrices obtained from the same body became evident, as will be shown in the following section.

3. Materials and methods

3.1. Samples obtained from the human body

Samples used in this work for determination and quantification of both cocaine and BZE were right cardiac blood (RCB or venous), left cardiac blood (LCB or arterial), femoral arterial blood (FAB), femoral venous blood (FVB), urine, VH, cerebrospinal fluid (CSF), brain accumbens nucleus (AN), brain ventral tegmental area (VTA), and liver. This spectrum of samples included both solid and liquid matrices, providing multiple possibilities to the coroner at time of sampling, as well as to the toxicologist at the time of analysis.

The diverse target matrices used to implement analytical methodologies in this study were obtained in the autopsy room from donors with no history of cocaine consumption. These records were collected in the usual interviews made to relatives of the deceased, as conducted at the Chilean Forensic Service. A screening