Rom J Leg Med [28] 398-402 [2020]
DOI: 10.4323/rjlm.2020.398

TOXICOLOGICAL STUDY OF AN EXHUMED BODY USING ALTERNATIVE MATRICES FOR THE DETECTION OF PSYCHOACTIVE DRUGS

Cristina Pérez-Martínez[1,*], Lucía Fernández-López[1], Gemma Prieto-Bonete[1], Aurelio Luna[1]

University of Murcia, School of Medicine, Regional Campus of International Excellence "Campus Mare Nostrum", Department of Legal and Forensic Medicine, Murcia, Spain

Abstract: In forensic toxicology, systematic toxicological analyses (STA) and interpretation of results are difficult when routine matrices such as blood (typically from a central and peripheral source), urine, bile, vitreous humor, stomach or gastric contents are not available. Furthermore, in instances of limited remains, only muscle, hair, or a skeleton may be available for toxicological examination to unambiguously identify as many toxicologically relevant compounds as possible to assist in determining the cause of death. This article describes a case of the exhumed corpse of a 53-year-old male known to have been addicted to several types of drugs. The STA was carried out on alternative biological matrices, including brain, kidney and bone marrow, using gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography–mass spectrometry (HPLC-MS) and subsequently the identified substances were quantified by GC-MS and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). The mixture found included multiple agents such as barbiturates, benzodiazepines (BZD) and anaesthetic agent. We demonstrate the possibility of detection of several drugs in an exhumed body 2 years after interment. Despite the problematic of interpreting the quantitative toxicological results, the data could provide complementary information about aetiology of death.

Keywords: forensic toxicology, systematic toxicological analyses, exhumed corpse, alternative biological matrices, gas chromatography–mass spectrometry, high performance liquid chromatography–mass spectrometry, ultra-performance liquid chromatography–tandem mass spectrometry, drugs.

INTRODUCTION

Forensic toxicology is aiming to detect possible xenobiotics, drugs and other potentially toxic compounds in biological autopsy samples, quantify the concentrations of relevant and main compounds found in samples, and contribute to the investigation of the manner of death [1, 2]. In order to contribute to the understanding and assessment of these test results in a medical-legal context [3], the use of appropriate analytical methods, tools, the technical knowledge of forensic toxicologists, etc. are necessary [4, 5]. In today's society, medical and recreational drugs represent most of the compounds that cause fatal poisonings [1].

The interpretation of the results of a toxicological analysis in post-mortem cases is complicated [6, 7], especially in the case of decomposing, poorly preserved, skeletonized bodies or corpses buried and later, and after a while, exhumed [8], due to the uncertainty of finding evidences after a given post-mortem interval (PMI) [9].

Peripheral and cardiac blood, urine, vitreous humour, bile, stomach or gastric contents are the typical biological samples collected at autopsy [10-13]. However, in cases where routine specimens cannot be analysed due to putrefaction or skeletonization [12] or in cases when forensic autopsy cannot collect adequate blood specimens [14], alternative matrices such as brain, kidney, liver, bone marrow and bone are useful for diagnostic purposes [3, 13]. But care must be taken, because high drug concentrations in blood may increase further during the post-mortem period, providing erroneous results: in such cases, the use of alternative specimens (such as brain, kidney, marrow bone, bone, etc.) will help improve forensic research and hopefully lead to a more complete understanding

*Correspondence to: Cristina Pérez-Martínez, PhD, University of Murcia, School of Medicine, Regional Campus of International Excellence "Campus Mare Nostrum", Department of Legal and Forensic Medicine, E-30100, Murcia, Spain, E-mail: cristina.perez.mtnz@gmail.com

of any drug-related event [3].

Besides variations in the type of sample, it has been found that the concentrations of drugs in tissues, organs or body fluids can vary significantly between death and the collection of the specimen [4]. Consequently, several factors must be considered such as the drug distribution and transformation in the perimortem and post-mortem states [15-23], the degradation of drugs, accidental or endogenous production and contamination, which can depend on the intake routes and the chemical properties of individual drugs and poisons [24-32]. All this makes it difficult to interpret post-mortem levels of drugs.

In any case, in addition to taking into account the type of sample, the conditions of the sample and the variations that may occur in the concentrations of the drugs, other factors must be considered such as the scope of the analyses, the suitability of the sample for the experiments, the stability or otherwise of the substances detected and other factors that influence the variation of the concentrations in the samples [6, 11, 33-34]. Therefore, despite obtaining positive results in the detection and quantifying drugs in biological samples obtained from an autopsy, it is complicated and sometimes not possible to interpret the results and extrapolate them to the antemortem state of the subject. This article reports the use of alternative matrices in post-mortem toxicological analysis in a case of suspected multi-drug abuse death of a patient with possible addiction habits.

CASE REPORT

A 53-year-old male was exhumed 2 years after death following circumstantial proof and evidence pointing to possible intentional intoxication. Despite the long burial period, the body was well preserved, although it was not possible to collect routine biological samples. Toxicological analyses were performed on bone marrow (20.1 g), brain (125 g) and kidney (48 g) at the Legal and Forensic Medicine Department of the University of Murcia (External Service for Forensic Science and Techniques (SECYTEF). Analysis of both illicit and therapeutic drugs were called for.

Materials, standards and chemicals

Based on the results obtained using GC-MS and HPLC-MS, the drugs with the highest toxicological relevance and deuterium-labeled standards were applied for Midazolam (M-908), Diazepam-D5 (D-902), Propofol-D17 (P-077) and Pentobarbital-D5 (P-009) were obtained from Cerilliant (Round Rock, TX). The analytical and chromatographic solvents used were acquired from Sigma Aldrich.

Standard and post-mortem samples preparation

Regression lines were assessed based on peak areas ratios of each of the 5 compounds to that of the standards. The method showed linearity, expressed by regression coefficient (R2) of above 0.975 in all cases. The LOQ was found to be between 0.005 and 0.01 µg/mL depending on the analyte, and the LOD was between 0.0008 and 0.05 µg/mL.

Duplicate biological post-mortem samples were homogenized using an IKA® ULTRA-TURRAX® disperser (Z404519, Sigma Aldrich). The homogenates were subjected to a drugs extraction procedure. The procedure described by Yawney *et al.* [35] was carried out in brain, kidney and liver post-mortem samples; while the protocol described by McIntyre *et al.* [36] was followed in case of the bone marrow samples. The extracts obtained from the processing of the samples were evaporated in nitrogen stream and reconstituted in 60 µl of mobile phase. Control and calibration standards were prepared by spiking methanol with standards at concentrations ranging from 0.005 µg/g to 5 µg/g.

Toxicological analysis

The screening of illicit and therapeutic drugs was performed by GC-MS and HPLC-MS after liquid-liquid extraction (LLE) and the addition of 25 µl of IS. The determination of concentrations of compounds such as propofol and pentobarbital was carried out by GC–MS and UPLC-MS/MS were used for midazolam. The toxins were confirmed by comparing the retention times and mass spectra characteristics of the peaks with those of standards and were quantified by means of external calibration curves.

RESULTS

General toxicological screening for common drugs performed in the alternative matrices available - bone marrow, brain and kidney - revealed the presence of the anaesthetic propofol, the barbiturate pentobarbital and BZD midazolam. These drugs were confirmed and quantified as shown in Table 1 and since the natural formation of this kind of compound can be ruled out, the observations clearly pointed to the antemortem intake of propofol, pentobarbital and midazolam.

Table 1. Summary of findings of toxicological analyses.

	Biological material		
Analyte	Bone marrow ppm (μg/g)	Brain ppm (μg/g)	Kidney ppm (μg/g)
Pentobarbital	0.2398	n.d.	0.0445
Propofol	n.d.	n.d.	0.8123
Midazolam	0.0055	0.2190	0.1050

n.d.: Not detected.

Our results evidenced of the existence of propofol at a concentration of 0.8123 μg/g in the kidney sample, which may be due to rapid redistribution and metabolism by conjugation in the liver to glucuronide and sulfate inactive metabolites, which are excreted by the kidney with elimination half-life of 23 hours [37].

The amount of pentobarbital found in the bone marrow sample was of 0.2398 μg/g and in kidney sample a concentration of 0.0445 μg/g. It was found in the kidney sample because is cleared by the kidneys [38] and its chemical stability, which allows it to be stored in the bone marrow. This also may reflect chronicity since it was not found in other samples, but further studies are needed to determine aspects such as the dose required and the frequency of use of the drug necessary for it to be deposited in the bone [39].

Midazolam was detected in three matrices, finding the highest concentration in brain (0.2190 μg/g) possibly due to its high lipophilicity at physiological pH, which explains for midazolam's rapid absorption and crossing of the blood-brain barrier [40, 41] BZDs and their metabolites are distributed throughout the body and accumulate mainly in lipid-rich areas such as adipose tissue and the central nervous system, where it exerts its action [42, 43]. On the other hand, we found this compound in lower concentrations in the kidney (0.1050 μg/g) coinciding with other study which reports that it finally goes to the excretory organs [44].

DISCUSSION

The tasks carried out by forensic sciences such as the identification and analysis of remains, especially when it comes to decomposed or incomplete bodies, is a complicated job [45]. Among the tests to be performed, we find the toxicological studies, which must be interpreted with great caution because the pharmacodynamic and kinetic properties of the compounds according to the different matrices are not clearly known. On the other hand, the concentrations found in biological samples will also depend on several factors, which will cause them to be modified, such as post-mortem redistribution [4], which is affected by cell death, decomposition, passive drug release from drug deposits after death [46], PMI, sample type, dissemination, redistribution in body cavities and drug metabolism after death [47, 48]. In addition, it is necessary to add the characteristics of the medications themselves - including lipid solubility, plasma protein binding and molecular size, all of which influence the volume of distribution [40].

Bibliographic references found on toxicological studies in non-conventional samples of exhumed corpses are scarce. The matrices used in these works have been putrefied material (abdominal area, muscles, etc.), skin, tissue, hair, nail, ulna and radius; the range of the PMI is between 10 days and 4.5 years and the compounds that had been detected are opiates, BZD, morphine and 6-acetylmorphine, and other types of drugs [8, 42, 49-51].

The most recent toxicological investigations carried out into accidental deaths, suicides, homicides and cases of drug-facilitated sexual assault involving the compounds identified in our case (propofol, pentobarbital and midazolam) showed higher concentrations in normal matrices such as femoral, cardiac and peripheral blood, head and pubic hair, urine and tissue samples of medico-legal autopsies [43, 52-71] than our results.

Interpretation of the toxicological results obtained in different biological matrices of an exhumed corpse 2 years after death is difficult because the processes of decomposition and putrefaction introduce substantial changes in the biological material.8 In addition to the changes in the matrices, we found substantial changes in the structure and composition of the drugs and toxicological problems arise as a consequence of the instability of the chemical in this type of situation [72-74]. Together with the absence of routine material (blood, urine or bile), this may lead to misinterpretation [72]. Despite these caveats, data from this study point to the relevance of toxicological analyses of alternative matrices to complement forensic investigation.

In conclusion, the detected substances, Midazolam, a type IV sedative agent of moderate action, would has a synergistic effect when administered with propofol (used as an intravenous

anesthetic in critical medical care) [75] and in addition, is incompatible with pentobarbital sodium, then, simultaneous administration could have caused a serious adverse reaction through an inhibitory effect on the cardiorespiratory centre [76], which it would be possible to establish a causal link between the death of the subject and substances found - midazolam, propofol and pentobarbital - at the concentrations mentioned.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Launiainen T, Ojanperä I. Drug concentrations in post-mortem femoral blood compared with therapeutic concentrations in plasma. Drug Test Anal. 2014; 6(4): 308-316.
2. Chamberlain J. The Analysis of Drugs in Biological Fluids, 2nd ed. CRC press. 1995; 1–33.
3. Caplan YH. Drug testing in alternate biological specimens. Springer Science & Business Media. 2008.
4. Han E, Kim E, Hong H, Jeong S, Kim J, In S, Chung H, Lee S. Evaluation of post-mortem redistribution phenomena for commonly encountered drugs. Forensic Sci Int. 2012; 219(1): 265-271.
5. Mußhoff F, Daldrup T, Aderjan R, Meyer L. Anlagen zu den Richtlinien zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen [Annexes to the Guidelines for Quality Assurance in Forensic Toxicological Investigations]. Toxichem. Krimtech 2002; 69: 32-34.
6. Drummer OH. Post-mortem toxicology. Forensic Sci Int. 2007; 165(2): 199-203.
7. Drummer OH, Gerastamoulos J. Post-mortem drug analysis: analytical and toxicological aspects. Ther Drug Monit. 2002; 24: 199–209.
8. Cippitelli M, Mirtella D, Ottaviani G, Tassoni G, Froldi R, Cingolani M. Toxicological analysis of opiates from alternative matrices collected from an exhumed body. J Forensic Sci. 2018; 63(2): 640-643.
9. Grellner W, Glenewinkel F. Exhumations: synopsis of morphological and toxicological findings in relation to the post-mortem interval: survey on a 20-year period and review of the literature. Forensic Sci Int. 1997; 90(1-2): 139-159.
10. Drummer OH, Kennedy B, Bugeja L, Ibrahim JE, Ozanne-Smith J. Interpretation of post-mortem forensic toxicology results for injury prevention research. Inj Prev. 2013; 19(4): 284-289.
11. Flanagan RJ, Connally G. Interpretation of analytical toxicology results in life and at post-mortem. Toxicol Rev. 2005; 24(1): 51-62.
12. Forrest ARW. Obtaining samples at post mortem examination for toxicological and biochemical analyses. J Clin Pathol. 1993; 48(4): 292-296.
13. Vardakou I, Athanaselis S, Pistos C, Papadodima S, Spiliopoulou C, Moraitis K. The clavicle bone as an alternative matrix in forensic toxicological analysis. J Forensic Leg Med. 2014; 22: 7-9.
14. Tominaga M, Michiue T, Ishikawa T, Kawamoto O, Oritani S, Ikeda K, Ogawa M, Maeda H. Post-mortem analyses of drugs in pericardial fluid and bone marrow aspirate. J Anal Toxicol. 2013; 37(7): 423-429.
15. Baselt RC. Disposition of toxic drugs and chemicals in man, 6th ed. Foster City, California: Chemical Toxicology Institute. Biomedical Publication. 2002.
16. Hilberg T, Ripel Å, Slørdal L, Bjørneboe A, Mørland J. The extent of post-mortem drug redistribution in a rat model. J Forensic Sci. 1999; 44(5): 956-962.
17. Hilberg T, Rogde S, Mørland J. Post-mortem drug redistribution-human cases related to results in experimental animals. J Forensic Sci. 1999; 44(1): 3-9.
18. Moriya F, Hashimoto Y. Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages post-mortem. J Forensic Sci. 1999; 44(1): 10-16.
19. Dalpe-Scott M, Degouffe M, Garbutt D, Drost M. A comparison of drug concentrations in post-mortem cardiac and peripheral blood in 320 cases. Can Soc Forensic Sci J. 1995; 28(2): 113-121.
20. Gomez HF, McKinney P, Phillips S, Roberts DV, Brent J, Watson WA. Post-mortem acetaminophen pharmacokinetics: an experimental study of site and time-dependent concentration changes. J Forensic Sci. 1995; 40(6): 980-982.
21. O'Sullivan JJ, McCarthy PT, Wren C. Differences in amiodarone, digoxin, flecainide and sotalol concentrations between antemortem serum and femoral post-mortem blood. Hum Exp Toxicol. 1995; 14(7): 605-608.
22. Shepherd MF, Lake KD, Kamps MA. Post-mortem changes and pharmacokinetics: review of the literature and case report. Ann Pharmacother 1992; 26(4): 510-514.
23. Prouty RW, Anderson WH. The forensic science implications of site and temporal influences on post-mortem blood-drug concentrations. J Forensic Sci. 1990; 35(2): 243-270.
24. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in post-mortem specimens: a review of the literature. Forensic Sci Int. 2007; 165: 10–29.
25. Schloegal H, Rost T, Schmidt W, Wurst FM, Weinmann W. Distribution of ethyl glucuronide in rib bone marrow, other tissues and body liquids as proof of alcohol consumption before death. Forensic Sci Int. 2006; 156: 213–218.
26. Iwasaki Y, Yashiki M, Namera A, Kojima T. On the influence of post-mortem alcohol diffusion from the stomach contents to the heart blood. Forensic Sci Int. 1998; 94: 111–118.
27. Sylverster PA, Wong NACS, Warren BF, Ranson DL. Unacceptably high site variability in post-mortem blood alcohol analysis. J Clin Path. 1998; 51: 250–252.
28. Singer PP, Jones GR. Very unusual ethanol distribution in a fatality. J Anal Toxicol. 1997; 21: 506–508.
29. Takayasu T, Ohshima T, Tanaka N, Maeda H, Kondo T, Nishigami J, Ohtsuji M, Nagano T. Experimental studies on post-mortem diffusion of ethano-d6 using rats. Forensic Sci Int. 1995; 76: 179–188.
30. Jones GR, Pounder DJ. Site dependence of drug concentrations in post-mortem blood - a case study. J Anal Toxicol. 1987; 11: 186–190.
31. Prouty RW, Anderson WH. A comparison of post-mortem heart blood and femoral blood ethyl alcohol concentrations. J Anal Toxicol. 1987; 11: 191–197.
32. Noguchi TT, Nakamura GR, Griesemer EC. Drug analyses of skeletonizing remains. J Forensic Sci. 1978; 23: 490–492.
33. Drummer OH. Forensic toxicology. Exs. 2010; 100: 579-603.
34. Skopp G. Preanalytic aspects in post-mortem toxicology. Forensic Sci Int. 2004; 142(2): 75-100.
35. Yawney J, Treacy S, Hindmarsh KW, Burczynski FJ. A general screening method for acidic, neutral, and basic drugs in whole blood using the Oasis MCX® column. J Anal Toxicol. 2002; 26(6): 325-332.
36. McIntyre IM, King CV, Boratto M, Drummer, OH. Post-mortem drug analyses in bone and bone marrow. Ther Drug Monit. 2000; 22(1): 79-83.
37. Marik PE. Propofol: therapeutic indications and side-effects. Curr Pharm Des. 2004; 10(29): 3639-3649.
38. Molina DK, McCutcheon JR, Rulon JJ. Head injuries, pentobarbital, and the determination of death. Am J Forensic Med Pathol. 2009; 30(1): 75-77.

39. McGrath KK, Jenkins AJ. Detection of drugs of forensic importance in post-mortem bone. Am J Forensic Med Pathol. 2009; 30(1): 40-44.
40. Griffin III CE, Kaye AM, Bueno FR, Kaye AD. Benzodiazepine pharmacology and central nervous system–mediated effects. Ochsner J. 2013; 13(2): 214-213.
41. Kothary SP, Brown AC, Pandit UA, Samra SK, Pandit SK. Time course of antirecall effect of diazepam and lorazepam following oral administration. Anesthesiology. 1981; 55(6): 641-644.
42. Guillot E, De Mazincourt P, Durigon M, Alvarez JC. Morphine and 6-acetylmorphine concentrations in blood, brain, spinal cord, bone narrow and bone after lethal acute or chronic diacetylmorphine administration to mice. Forensic Sci Int. 2007; 166: 139–144.
43. Kirby RR, Colaw JM, Douglas MM. Death from propofol: accident, suicide, or murder? Anesth Analg. 2009; 108(4): 1182-1184.
44. Oertel R, Pietsch J, Arenz N, Zeitz SG, Goltz L, Kirch W. Distribution of metoprolol, tramadol, and midazolam in human autopsy material. J Chromatogr A. 2011; 1218(30): 4988-4994.
45. Delabarde T, Keyser C, Tracqui A, Charabidze D, Ludes B. The potential of forensic analysis on human bones found in riverine environment. Forensic Sci Int. 2013; 228(1): e1-e5.
46. Yarema MC, Becker CE. Key concepts in post-mortem drug redistribution. Clin Toxicol. 2005; 43(4): 235-241.
47. Staeheli SN, Baumgartner MR, Gauthier S, Gascho D, Jarmer J, Kraemer T, Steuer AE. Time-dependent post-mortem redistribution of butyrfentanyl and its metabolites in blood and alternative matrices in a case of butyrfentanyl intoxication. Forensic Sci Int. 2016; 266: 170-177.
48. Kennedy MC. Post-mortem drug concentrations. Intern Med J. 2010; 40(3): 183-187.
49. Chaudhary MT, Wattoo SA, Sarwar M, Tahir MA, Imran M, Ashiq, MZ. Toxicological Analysis of Exhumed Specimen-Challenge for the Toxicologists. Arab Journal of Forensic Sciences & Forensic Medicine. 2016; 1(3); 354-361.
50. Bardale R, Ambade V, Dixit P. Exhumation: A 10-year retrospective study. J Indian Acad Forensic Med. 2012; 34(2); 143-145.
51. Karger B, Lorin de la Grandmaison G, Bajanowski T, Brinkmann B. Analysis of 155 consecutive forensic exhumations with emphasis on undetected homicides. Int J Legal Med. 2004; 118: 90–94.
52. Klausz G, Róna K, Kristóf I, Törő K. Evaluation of a fatal propofol intoxication due to self administration. J Forensic Leg Med. 2009; 16(5): 287-289.
53. Kranioti EF, Mavroforou A, Mylonakis P, Michalodimitrakis M. Lethal self administration of propofol (Diprivan): a case report and review of the literature. Forensic Sci Int. 2007; 167(1): 56-58.
54. Roussin A, Mirepoix M, Lassabe G, Dumestre-Toulet V, Gardette V, Montastruc JL, Lapeyre-Mestre M. Death related to a recreational abuse of propofol at therapeutic dose range [Correspondence]. Br J Anaesth. 2006; 97(2): 268.
55. Strehler M, Preuss J, Wollersen H, Madea B. Lethal mixed intoxication with propofol in a medical layman. Arch Kriminol. 2006; 217(5-6): 153-160.
56. Iwersen-Bergmann S, Rösner P, Kühnau HC, Junge M, Schmoldt A. Death after excessive propofol abuse. Int J Legal Med. 2001; 114(4-5): 248-251.
57. Chao TC, Lo DST, Chui PPS, Koh TH. The first fatal 2, 6-di-isopropylphenol (propofol) poisoning in Singapore: a case report. Forensic Sci Int. 1994; 66(1): 1-7.
58. Drummer OH, A fatality due to propofol poisoning. J Forensic Sci. 1992; 37(4): 1186-1189.
59. Cirimele V, Kintz P, Doray S, Ludes B. Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. Int J Legal Med. 2002; 116(1): 54-57.
60. Melo P, Costa P, Quintas MJ, Castro A, Tarelho S, Franco JM, Teixeira HM. Pentobarbital in the context of possible suicides: Analysis of a Case. Forensic Sci Int. 2017; 274: 109-112.
61. Hangartner S, Steiner J, Dussy F, Moeckli R, Gerlach K, Briellmann T. A suicide involving intraperitoneal injection of pentobarbital. Int J Legal Med. 2016; 5: 1217-1222.
62. Cantrell FL, Nordt S, Mcintyre I, Schneir A. Death on the doorstep of a border community–intentional self-poisoning with veterinary pentobarbital. Clin Toxicol. 2010; 48(8): 849-850.
63. Brandt-Casadevall C, Krompecher T, Giroud C, Mangin P. A case of suicide disguised as natural death. Sci Justice. 2003; 43(1): 41-43.
64. Romain N, Giroud C, Michaud K, Mangin P. Suicide by injection of a veterinarian barbiturate euthanasia agent: report of a case and toxicological analysis. Forensic Sci Int. 2003; 131(2): 103-107.
65. Poklis A, Hameli AZ. Two unusual barbiturate deaths. Arch Toxicol. 1975; 34(1): 77-80.
66. Frison G, Favretto D, Tedeschi L, Ferrara SD. Detection of thiopental and pentobarbital in head and pubic hair in a case of drug-facilitated sexual assault. Forensic Sci Int. 2003; 133(1): 171-174.
67. Dordevic S, Tomasevic G, Kilibarda V. Fatal Overdose with Midazolam-Application of HPLC-PDA Method. Med Data Rev. 2010; 2(3): 251-255.
68. Nishiyama T, Hanaoka K. Accidental overdose of midazolam as intramuscular premedication. J Clin Anesth. 2002; 14(7): 543-555.
69. Michalodimitrakis M, Christodoulou P, Tsatsakis AM, Askoxilakis I, Stiakakis I, Mouzas I. Death related to midazolam overdose during endoscopic retrograde cholangiopancreatography. Am J Forensic Med Pathol. 1999; 20(1): 93-97.
70. Yashiki M, Miyazaki T, Iwasaki Y, Taniguchi T, Kozima T. A case of suicide by an intravenous injection of pancuronium [in Japanese]. Nippon Hagaku Zasshi. 1992; 46(4): 282-285.
71. Ferslew KE, Hagardorn AN, McCormick WF. Post mortem determination of the biological distribution of sufentanil and midazolam after an acute intoxication. J Forensic Sci. 1989; 34(1): 249-257.
72. Kaferstein H, Stich G, Madea B. Chlorprothixene in bodies after exhumation. Forensic Sci Int. 2013; 229: 30–34.
73. Drummer OH. Post-mortem toxicology of drugs of abuse. Forensic Sci Int. 2004; 142: 101–113.
74. Moriya F, Hashimoto Y. Post-mortem stability of cocaine and cocaethylene in blood and tissues of humans and rabbits. J Forensic Sci. 1996; 41: 612–616.
75. Tominaga M, Michiue T, Ishikawa T, Kawamoto O, Oritani S, Ikeda K, Maeda H. Post-mortem analyses of drugs in pericardial fluid and bone marrow aspirate. J Anal Toxicol. 2013; 37(7): 423-429.
76. Agencia española de medicamentos y productos sanitarios (AEMPS). Ficha Técnica Midazolam. https://www.aemps.gob.es/cima/pdfs/es/ft/64092/P_64092.pdf; 2009 Accessed 29 November 2017.