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# Comparison of post-mortem xenobiotics in blood, brain and bone marrow

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**Abstract:** The bone marrow is the tissue contained in the center and in the epiphysis of the bones and represents the site where new blood cells are produced. It is one of the potentially alternative tissues in forensic toxicology. It could be particularly useful in that cases with no availability of the usual sample due to advanced state of decomposition or to skeletonization of the corpse. So far, such tissue has been used for the genetic and histopathological post-mortem diagnosis of several malignant pathologies, whilst poor toxicological data about drug distribution or incorporation are available. In the present study, we investigated seven lethal intoxications using blood, brain and rib bone marrow samples collected during autoptic exams and analyzed by UHPLC-MS/MS. The drug concentrations in the bone marrow were lower than the blood and brain ones. The toxicological interpretation of the data is difficult because of the lack of literature about this subject, so that further studies should be conducted.

**Key Words:** Postmortem bone, bone marrow, Drugs of abuse, UHPLC-MS/MS analysis, Toxicology.

## INTRODUCTION

It is well known that, after death, the body is subjected to decomposition and often such processes make it impossible to collect usual biological samples. Even if blood is the elective sample for qualitative and quantitative toxicological investigations, several studies suggest to use alternative matrices, such as bone tissue, in cases of skeletal or decomposed remains, where no usual sample is available [1-3].

The concentration of drugs in this matrix can be affected by many factors, such as chronic or acute consumption, the distribution at the time of death, the site of bone sampling, the type of bone tissue and the physico-chemical characteristics of the drugs [4].

Toxicological investigations have been focused always on the analyses of biological liquids (blood, vitreous humor, urine, bile and gastric contents) and of certain organs, such as brain, lung, liver and kidney. However, since bone and bone marrow represent about 14% of the body mass [5-6], it can be hypothesized that such tissues could be a temporary deposit site of substances due to it is a well-vascularized tissue and has an high quantity of lipids. Other advantage is that the bone is protected from external contamination [1, 4, 6].

Several studies have highlighted the potential of bone and bone marrow as an alternative matrix for forensic analysis [7-8]. The first toxicological study about bone marrow, concerning the kinetics of alcohol, dates back to 1943 [7, 9]. Such tissue is also used in genetic [7-8] and histopathological analysis for post-mortem diagnosis of several malignant pathologies. The study was carried out on rib fragments considering the easy of sampling and its large amount of bone marrow.

The aim of the present study is to determination of the concentrations in rib of toxic substances and compare these concentration with those of other tissue (blood and brain).

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## MATERIALS AND METHODS

### *Chemicals and reagents*

Methanol LC–MS grade was purchased from Fisher Scientific International Inc. (Hampton, NH, USA) and formic acid (>98%) was purchased from Riedel-de Haën® (Sigma–Aldrich, Steinheim Germany). HPLC grade water was obtained by Milli-Q purification system. Compounds of the highest available purity were used as standards and internal standard were supplied by Lipomed (Arlesheim, Switzerland) and Cerilliant (Texas, USA). Cocaine-D3 was used as internal standards (IS) in a concentration of 100 ng/g of bone.

### *Preparation of calibration standards*

Individual stock solutions of the 8 compounds of interest were prepared by dissolving drugs in absolute methanol to obtain final drug concentrations of 1 mg/mL. Stock solutions were stored at −20 °C. Standard solutions for calibration curves and quality control were prepared by further dilution of the corresponding stock solution with methanol to 100 μg/mL, 10 μg/mL, 1 μg/mL and 0.1 μg/mL. All solutions were stored at −20 °C. Calibration standards were prepared by spiking drug-free human rib at seven concentration levels (5, 50, 100, 500, 1000, 2000 and 5000 ng/g of rib).

### *Samples*

The samples were collected in autopsy, 24–48 h post mortem, where a small piece of the rib (about 5 cm of length, sampled in the middle of the eighth rib), the femoral blood and brain were taken for our study. All of them belonged to men with confirmed history of taking pharmaceuticals and illicit drugs. In total 21 samples were studied from 7 individuals.

### *LC–MS/MS instrumentation and conditions*

Chromatography was performed by an ACQUITY UPLC System –(Waters Corporation) on an Acquity BEH C18 column (150 × 2.1 mm i.d., 1.7 μm; Waters) protected by an Acquity BEH C18 VanGuard pre-column (5 mm × 2.1 mm i.d., 1.7 μm; Waters) using a mobile phase of A: water, 0.1% formic acid over B: methanol, 0.1% formic acid. MS/MS detection of analytes and IS was carried out on a Xevo TQD system (Waters, UK) with electrospray ionization operating in positive and in negative mode on switching mode. Argon was used as the collision gas for the MS/MS experiments. MRM mode was applied for the detection and quantification of all 8 analytes of interest by monitoring selected transitions. Capillary voltage was set at +3.5 kV, source and dessolvation temperatures were set at 150 °C and 350 °C respectively while dessolvation gas flow was 650 L/h and cone gas flow 50 L/h. Cone voltage and collision energy were optimized for each transition. Data acquisition and analysis was performed by MassLynx® software whereas quantitation was performed by TargetLynx application [11].

### *Extraction*

For extraction of the rib, it was used the validated method of Orfanidis *et al.* (2018) [11]. In brief, each bone was cleaned until any remains of muscles or other connective tissues were removed meticulously using a scalpel. After they was washed with deionized water to remove external contaminations (soil etc.). Then the rib was left to dry in the air. Afterwards the ribs were ground using a mortar. The crushed bones were stored at −20 °C until analysis.

Frozen bone samples were left reach room temperature. Then 1 g of bone was weighed and 3 mL of MeOH and 12.5 μL of NH4OH (13.4 M) were added to adjust the pH at 10. After that, the mixture was rotated for 5 h and was put at ultrasonic bath for 1 h. Then it was centrifuged at 10.000 rpm for 10 min and after filtration (0.22 μm) to remove bone remains the supernatant was transferred in another bottle. The supernatant was evaporated to dryness under a steam of nitrogen. The residue was reconstituted in 300 μL (H2O: MeOH – 80:20 v/v), filtrated (0.22 μm) again and thereafter transferred into autosampler vial for analysis [11].

For brain and blood extractions, it was used the method of Goldberger *et al.* [12] for cocaine, opioids and alkaloids compound. Instead, for extraction of other drugs, it was used the direct extraction method with chlorobutane as reported in Clarke's [13].

## RESULTS

The results of the toxicological analyzes of the blood and brain samples and on the segments of rib about the cases examined are reported in the following tables. The same tables indicate the concentrations (Table 1a - 1g).

## DISCUSSION

The rib can be a potentially useful alternative biological matrix in forensic cases where blood or other usual tissues are not available, since the abundant vascularization, the large lipid amount and the protection from possible contamination due to the surrouding presence of bone tissue. It is reasonable to presume that bone marrow could represent a temporary depository for drugs and their metabolites. Indeed, the mechanisms of incorporation and storage in inorganic bone tissue are uncertain because of the wake affinity with the organic substances and data of literature are too limited. The assessment of the degree of correlation of the individual xenobiotic concentration in bone marrow and blood was initially studied by Winek *et al.* (1982) [3]. Currently, no data are available about correlation among blood, brain and bone morrow.

In the present study, seven lethal intoxication cases were investigated by the analysis of blood, brain and rib tissue samples collected during the autopsy exams.

The same substances were found both in the ribs and in the blood and brain. However, although psychotropic drugs have conceivably high affinity with the lipid part of the bone marrow, we found them at lower concentrations. It is unclear whether it depends on the used method, or on the variable and uncertain ratio between the rib sample and the bone marrow contained in it, or on the lower drug bone marrow distribution/incorporation (Table 2).

On the basis of our results, only recently consumed drugs seems well detectable, whereas it's not clear whether remote consumption related substances can be found. In fact, the cases nn. 1 and 2 are suicides by acute intoxications of large amounts of drugs in non-habitual consumers, whilst the other cases are lethal overdoses of inveterate drug addicts.

Since there is no data available about distribution of drugs in bone marrow, further studies on this subject should be required.

***Table 1a***

| Case n. 1 | Blood | Brain | Rib tissue |
|---|---|---|---|
| EDDP | 122.1 ng/mL | 283.3 ng/g | 61.8 ng/g |
| Methadone | 631.5 ng/mL | 815.2 ng/g | 387.2 ng/g |

***Table 1b***

| Case n. 2 | Blood | Brain | Rib tissue |
|---|---|---|---|
| Promazine | 176.1 ng/mL | 372.9 ng/g | 63.5 ng/g |

***Table 1c***

| Case n. 3 | Blood | Brain | Rib tissue |
|---|---|---|---|
| Cocaine | 415.4 ng/mL | 1.331.1 ng/g | 95.3 ng/g |
| Benzoylecgonine | 1.644.6 ng/mL | 215.4 ng/g | 189.5 ng/g |

***Table 1d***

| Case n. 4 | Blood | Brain | Rib tissue |
|---|---|---|---|
| Codeine | 19.4 ng/mL | 29.3 ng/g | 24.7 ng/g |
| Morphine | 327.1 ng/mL | 272.4 ng/g | 285.4 ng/g |
| 6-MAM | N.Q. | 53.2 ng/g | N.Q. |

***Table 1e***

| Case n. 5 | Blood | Brain | Rib tissue |
|---|---|---|---|
| Cocaine | 514.2 ng/mL | 1.631.8 ng/g | 129.9 ng/g |
| Benzoylecgonine | 2.143.6 ng/mL | 417.8 ng/g | 203.7 ng/g |

***Table 1f***

| Case n. 6 | Blood | Brain | Rib tissue |
|---|---|---|---|
| Cocaine | 296.7 ng/mL | 947.8 ng/g | 187.8 ng/g |
| Benzoylecgonine | 963.1 ng/mL | 184.7 ng/g | 197.8 ng/g |

***Table 1g***

| Case n. 7 | Blood | Brain | Rib tissue |
|---|---|---|---|
| Codeine | 63.7 ng/mL | 112.3 ng/g | 47.1 ng/g |
| Morphine | 564.7 ng/mL | 421.5 ng/g | 214.4 ng/g |
| 6-MAM | 83.2 ng/mL | 121.8 ng/g | N.Q. |

**Table 2.** Description of the cases

| | Sex | Age | Researched substances | *Causa mortis* |
|---|---|---|---|---|
| **Case n. 1** | M | 41 | Methadone, EDDP | Suicide |
| **Case n. 2** | M | 48 | Promazine | Suicide |
| **Case n. 3** | F | 49 | Cocaine, Benzoylecgonine | Overdose |
| **Case n. 4** | M | 38 | Morphine, Codeine, 6-MAM | Overdose |
| **Case n. 5** | M | 51 | Cocaine, Benzoylecgonine | Overdose |
| **Case n. 6** | F | 48 | Cocaine, Benzoylecgonine | Overdose |
| **Case n. 7** | F | 39 | Morphine, Codeine, 6-MAM | Overdose |

**Conflict of interest.** The authors declare that there is no conflict of interest.

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