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ORIGINAL ARTICLE

EARLY PHASE POSTMORTEM REDISTRIBUTION OF LIDOCAINE AND MIDAZOLAM IN ADULT ALBINO RATS

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ABSTRACT

Background: Postmortem redistribution (PMR) is the changes that occur in drug concentrations after death. Lidocaine is the most popular local anesthetic used worldwide and midazolam is a widely used pre-anesthetic anxiolytic and sedative. **Aim of the Work:** This work was performed to study potential early phase postmortem redistribution of lidocaine and midazolam, as well as, the influence of storage temperature on it in adult albino rats. This was done by measuring their concentrations in blood (cardiac blood and external iliac vein blood) and tissues (heart, lungs and liver). Calculation of cardiac blood to peripheral blood ratio (C/P) and Liver to peripheral blood ratio (L/P) was performed. **Materials and Methods:** This study was carried out on 36 adult male albino rats which divided into two main groups (18 rats each). **Group I (Lidocaine)**: Rats received a single SC injection of 2% lidocaine HCL (67 mg/kg), and sacrificed 30 minutes later. This group was subdivided into three equal groups; AM control **(L-AM)**, 15 minutes PM at 4ºC **(L-PM4)** and 15 minutes PM at 21ºC **(L-PM21)**. **Group II (Midazolam)**: Rats received single IV injection of midazolam (75 mg/kg), and sacrificed 30 minutes later. This group was subdivided into three equal groups; AM control **(M-AM)**, 15 minutes PM at 4ºC **(M-PM4)**, 15 minutes PM at 21ºC **(M-PM21)**. **Results:** There were significant changes in lidocaine and midazolam concentrations in both tissues and blood samples as compared to those of corresponding AM control groups. Markers of PMR revealed early phase PMR of lidocaine by L/P ratios > 20 at 21 ºC. Storage temperature at 4ºC arrested lidocaine PMR as recorded by both C/P ratios < 1 and L/P ratios < 5. Midazolam was prone to postmortem degradation that interfered with PMR assessment. Midazolam revealed minimal early phase postmortem redistribution as demonstrated by C/P ratios just above 1 at 4 ºC. L/P ratio was a more reliable marker for PMR than C/P ratio. **Conclusion:** Lidocaine was highly liable to undergo early phase PMR as demonstrated by L/P ratios above 20 at 21 ºC. However, storage at 4ºC retarded lidocaine PMR. Midazolam was subjected to postmortem degradation and had minimal early phase PMR as demonstrated by C/P ratios just above 1 at 4 ºC. **Recommendation:** it is recommended to increase forensic toxicologists' awareness about PMR of lidocaine and midazolam, and their influence on the interpretation of PM toxicological analysis.

Key Words: PMR; lidocaine; midazolam; storage temperature; GCMS; C/P ratio and L/P ratio.

INTRODUCTION

Drug blood concentrations are frequently used in forensic science, besides scene evidence and autopsy data, in identifying the possible cause and manner of death. Postmortem redistribution (PMR) is a significant issue that complicates postmortem drug analysis. This phenomenon is referred to the variations that occur in drug concentrations after death. It implies the movement of drugs into blood from solid organs e.g. lungs, liver and heart **(Özşeker et al., 2015)**.

Although postmortem drug concentrations in blood may not always reflect the antemortem levels, these concentrations may adapt some accepted trends that assist in their interpretation. The mechanisms engaged in PMR are still not very known, but the characteristics of the drug itself are very useful in predicting its liability for redistribution after death **(McIntyre, 2015)**. PMR is predicted for basic lipophilic drugs with volume of distribution greater than 3 L/kg. These drugs exhibit higher levels in blood collected from central body cavity and heart than blood that is

collected from peripheral sites. A possible explanation of this phenomenon is the diffusion of drugs from organs **(McIntyre, 2014)**.

Postmortem drug concentrations in peripheral blood were assumed to be fairly equivalent to their antemortem levels. Accordingly, blood samples obtained from peripheral sites are recommended for postmortem drug analysis. Cardiac to peripheral blood (C/P) concentration ratio is used by many laboratories in assessing the redistribution of drugs in postmortem cases. Since postmortem drug concentration is more stable in tissues, liver to peripheral blood (L/P) concentration ratio has been suggested as a more reliable marker for determining PMR **(Özşeker et al., 2015)**

Regional anesthesia has become a new trend that is widely used by anesthesiologists to provide intraoperative anesthesia, and postoperative analgesia **(Lirk and Hollmann, 2014)**. Unfortunately, systemic toxicity may result from accumulation of local anesthetics in blood, mostly after unintentional intravascular injections. However, systemic overdoses can be accounted for the excessive amounts injected in any anatomical site. This toxicity is typically presented by neurologic or cardiovascular manifestations **(Anderson et al., 2015).**

Lidocaine is the most popular local anesthetic used worldwide, and is considered one of the safest and most effective local anesthetics ever manufactured. It has been in clinical use for over 60 years, since it was first marketed in 1948 **(Dillane and Finucane, 2010)**. Local anesthesia is regularly applied to skin, subcutaneous tissue, and periosteum in minor operations and invasive investigative procedures. For anxious patients, the use of analgesic and/or tranquilizer is favorable. This approach enhances the ability of the patient to tolerate the procedure, and makes him more comfortable and cooperative **(Park et al., 2008)**.

Benzodiazepines (BNZ) are considered one of the most popular anxiolytic drugs used in clinical practice. They are indicated for treatment of anxiety disorder and panic disorders, sedation, light anesthesia and anterograde amnesia of perioperative events, control of seizures, and skeletal muscle relaxation **(Iqbal et al., 2002)**. In acute overdose, BNZ are much less hazardous than other anxiolytic/hypnotic medications, as they produce prolonged sleep without serious respiratory or cardiovascular depression. In spite of that, presence of other CNS depressants, especially alcohol, cause severe life-threatening respiratory depression, particularly when BNZ are abused. Moreover, the advantage of presence of flumazenil as an effective antagonist in acute overdoses is not available for most CNS depressants **(Rang et al., 2014).**

Midazolam is a short-acting BNZ, which has potential advantages over diazepam as it is roughly 1.5-2 times more potent, and it has a stronger hypnotic effect than diazepam due to its interference with GABA reuptake. Moreover, it has less venous irritation and less thrombophlebitis than diazepam **(Griffin et al., 2013)**. Consequently, midazolam is the most commonly used agent to decrease preoperative anxiety which is an unpleasant state of uneasiness or tension in patients being concerned about a disease, hospitalization, anesthesia, and surgery, or the unknown **(Patel & Kurdi, 2015)**.

Aim of the Work: The aim of this study was to estimate the early phase postmortem redistribution of lidocaine and midazolam, as well as, the influence of storage temperature on it in adult male albino rats.

MATERIAL AND METHODS

A- Material:

Chemicals:

Lidocaine Hydrochloride (2% Lidocaine HCL): Used as 50 ml vial contains 20 mg/ml lidocaine HCL; the Arab Company for Gelatin and Pharmaceutical Products, Egypt. **Midazolam:** Used as ampoules each contains 5 mg/ml midazolam; Roche Pharmaceutical Company, Switzerland. **Reagents:**They were obtained from Sigma/Aldrich Company, USA and Biodiagnostic Company, Egypt.

Animals:

Adult albino rat was the animal of choice for this study because of its metabolic proximity with human **(Johnson, 2007)**. The study was carried out on 36 adult male albino rats weighing 200-250 g; they were obtained from Faculty of Veterinary Medicine, Zagazig University. Before starting the experiment, all animals were subjected to 2 weeks of passive preliminaries for house acclimatization, to ascertain their physical well-being and to exclude any diseased animal.

The Institutional Review Board (IRB) committee for scientific research of Faculty of Medicine, Zagazig University approved the design of the experiment. All animals received human care in compliance with the Animal Care Guidelines and Ethical Regulations in accordance with "The Guide for the Care and Use of Laboratory Animals" **(Institute of Laboratory Animal Resources, 1996)**.

Study design:

The study has been designed in the Faculty of Medicine, Zagazig University. This study was designed to mimic an acute intoxication in humans, dosages chosen for both drugs represent 1/5 LD50 for rats {lidocaine HCL SC LD50 is 335 mg/kg, while midazolam IV LD50 is 75 mg/kg **(Lewis, 2004)**}. After housing acclimatization, rats were divided into two main groups; Lidocaine and Midazolam (18 rats in each). Each group was further subdivided into 3 subgroups (6 rats each).

Group I (Lidocaine):

Rats received a single subcutaneous SC injection of 2% lidocaine HCL (67 mg/kg), and then were sacrificed 30 minutes later. This group was further subdivided according to time and temperature at which blood and tissue samples were collected into; **L-AM (control):** Blood and tissue samples were collected under anesthesia immediately before scarification for determination of antemortem concentrations. **L-PM4:** After scarification, rats were left lying on their backs for 15 minutes at 4ºC in a refrigerator prior to autopsy. **L-PM21:** After scarification, rats were left lying on their backs for 15 minutes at 21ºC conditioned room temperature prior to autopsy **(Kugelberg et al., 2005).**

Group II (Midazolam):

Rats were received single intravenous IV injection of midazolam (75 mg/kg), and then rats sacrificed 30 minutes later. This group was further subdivided according to time and temperature at which blood and tissue samples were collected into: **M-AM (control):** Blood and tissue samples were collected under anesthesia immediately before scarification for determination of antemortem concentrations. **M-PM4:** After scarification, rats were left lying on their backs for 15 minutes at 4ºC in a refrigerator prior to autopsy**. M-PM21:** After scarification, rats were left lying on their backs for 15 minutes at 21ºC conditioned room temperature prior to autopsy **(Kugelberg et al., 2005).**

B-Methods:

1-Sample preparation:

Blood samples collection and extraction:

Peripheral blood was collected from the external iliac vein and cardiac blood by direct puncture after clamping the inferior vena cava just above the diaphragm and placed in sodium fluoride tubes **(Castaing et al., 2006)**.

A liquid–liquid extraction procedure was used for blood samples preparation. 0.5 mL of blood and 0.5 mL of distilled water were added to a glass tube. Then 1 mL of 1% HCL was added to the glass tube. After mixing for 15 minutes, the tube was centrifuged at 3000 rpm for 15 minutes using eppendorf centrifuge 5430R (*eppendorf AG 2331 Hamburg, Germany)*, and the supernatant acid was transferred to a glass tube. Then, 1 mL of 0.5 M NaOH and 2.5 mL of diethyl ether were added and the diethyl ether layer was evaporated to dryness in an evaporating dish. The residue was dissolved in 500 µl of ethanol of which 2 µl of the solution was injected into the Gas Chromatography–Mass Spectrometry (GC-MS) **(Zhang et al., 2015)**.

Tissue samples collection and extraction:

After collection of whole blood samples; heart, lungs and liver were also collected and wrapped in aluminum foil. Each sample were

immediately frozen and stored at -20 ºC until used for drug extraction and quantitative analysis via GC-MS **(Castaing et al., 2006)**.

A liquid–liquid extraction procedure was used for tissues samples preparation. 0.5 g of tissue and 0.5 mL of distilled water were homogenized intensively using tissue homogenizer (*Janke & kunke, GMBH & CO.KG, IKA®Labortechnik, D7813 stautent Br., ultra-Turrax T25; 220V, 600 W, 24000 min^{-1})* and the matter was transferred to a glass tube. Then 1 mL of 1% HCL was added to the glass tube. After mixing for 15 minutes, the tube was centrifuged at 3000 rpm for 15 minutes using eppendorf centrifuge 5430R (*eppendorf AG 2331 Hamburg, Germany)*, and the supernatant acid was transferred to a glass tube. Then, 1 mL of 0.5 M NaOH and 2.5 mL of diethyl ether were added and the diethyl ether layer was evaporated to dryness in an evaporating dish. The residue was dissolved in 500 µl of ethanol of which 2 µl of the solution was injected into the GC-MS **(Zhang et al., 2015)**.

2- Gas Chromatography–Mass Spectrometry (GC-MS) Analysis:

Trace GC Ultra-TSQ Mass Spectrometer *(Thermo Scientific, Austin, TX, USA located at Doping laboratory, International Medical Center, Cairo, Egypt)* was used to determine lidocaine and midazolam in tissues and blood samples. The GC-MS conditions were as following:

Lidocaine group samples:

The column was capillary TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 100°C hold for 1 minute and then increased by 35°C /min to 280°C and increased by 60°C /min to 300 °C hold for 2 minutes. The injector and MS transfer line temperatures were kept at 220 and 250°C respectively, Helium was used as a carrier gas at a constant flow rate of 1.2 ml/min. Samples of 2 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. The ion source was set at 200 °C. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–400 in full scan mode. Selected ion monitoring (SIM) in one segment was used for quantitative analysis of lidocaine. SIM ions were 56, 66,217 and 234. The Lidocaine compound was identified in the samples by comparison of their retention times and mass spectra with those of NIST 11 mass spectral database.

Quantitative analysis was done via external standardization method using a calibration curve made using serially diluted solutions containing known specified amounts of lidocaine as standards; 0.01ug/ml, 0.1 ug/ml, 1 ug/ml, 10 ug/ml and 100 ug/ml (Fig. 1).

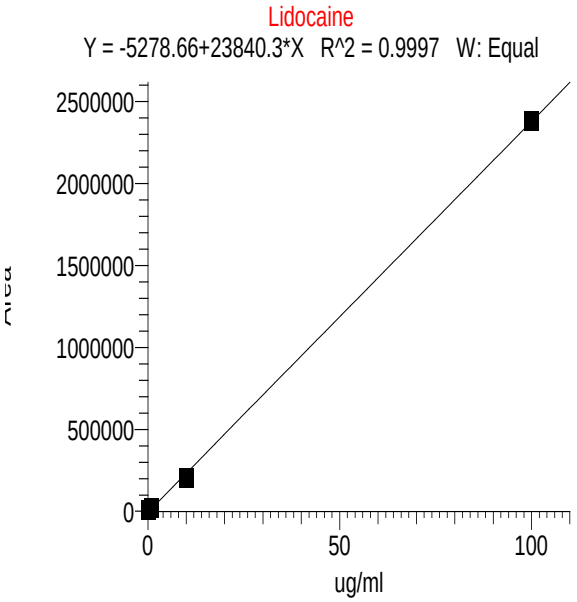

Fig. (1): Calibration graph for quantitative analysis of lidocaine in blood and tissues by GC-MS

Midzolam group samples:

The column was capillary TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 100°C hold for 1 minute and then increased by 35°C /min to 280°C and increased by 60°C /min to 300°C hold for 2 minutes. The injector and MS transfer line temperatures were kept at 220 and 250°C respectively, Helium was used as a carrier gas at a constant flow rate of 1.2 ml/min. Samples of 2 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. The ion source was set at 200 °C. EI mass spectra were collected at 70 eV ionization voltages over the

range of m/z 50–350 in full scan mode. Selected reaction monitoring (SRM) in one segment was used for quantitative analysis of midzolam SRM parent masses were 297.150, 310.100, 310.102, 310.104, 311.200, 311.204, 311.205, 325.000, and 325.010. The midzolam compound was identified in the samples by comparison of their retention times and mass spectra with those of NIST 11 mass spectral database.

Quantitative analysis was done via external standardization method using a calibration curve made using serially diluted solutions containing known specified amounts of midazolam as standards; 0.5 ug/ml, 1 ug/ml, 10 ug/ml, 50 ug/ml and 100 ug/ml (Fig. 2).

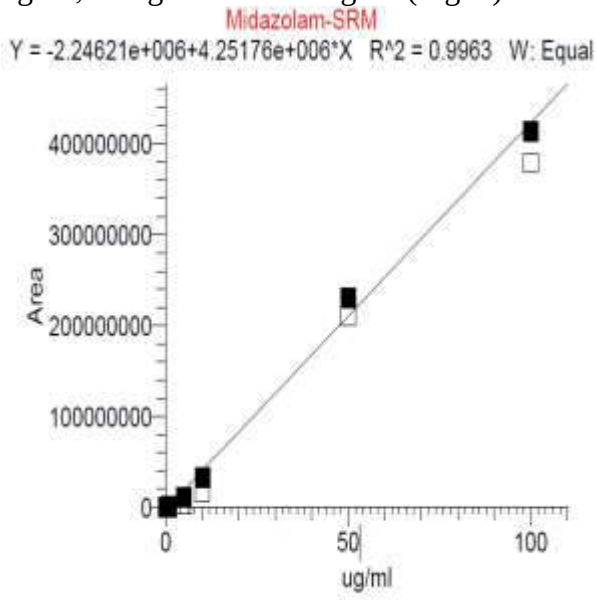

Fig. (2): Calibration graph for quantitative analysis of midazolam in blood and tissues by GC-MS

RESULTS

A- Chromatographic results:

I- Lidocaine group:

Blood extracts of the studied antemortem control group (L-AM) showed that lidocaine concentration in peripheral blood was greater than cardiac blood. Tissue extracts revealed that heart was the main reservoir of lidocaine followed by lung and liver respectively. Generally, lidocaine had greater concentrations in tissues than blood, whether cardiac blood or peripheral blood (Fig.3).

When compared to L-AM control group, lidocaine blood concentrations in 15 minutes postmortem at 4°C (L-PM4) showed a non-significant increase in blood, both cardiac (P >0.05) and peripheral (P >0.05). On the contrary, tissues showed a significant decline in heart and lung concentrations, which was markedly observed in heart that almost halved its lidocaine concentration with high significance (P <0.0001), also lung showed a significant decrease (P <0.01). However, liver demonstrated a non-significant decrease (P >0.05) (Table 1, Fig. 4).

When compared to L-AM control group, lidocaine blood concentrations in 15 minutes postmortem at 21°C (L-PM21) revealed a significant increase in blood, both cardiac (P <0.05) and peripheral (P <0.01). Meanwhile, heart revealed a high significant decrease in heart concentration (P<0.0001). However, liver and lung concentrations displayed a significant rise in their lidocaine concentrations (P <0.0001 and P <0.001, respectively) (Table 2, Fig. 5).

Lidocaine peaks were detected in chromatograms of antemortem control L-AM and postmortem groups, L-PM4 and L-PM21 at retention time (RT) = 4.46 – 4.66 (Figs. 6, 7 and 8).

II- Midazolam groups:

In antemortem control group (M-AM) midazolam concentration in blood extracts illustrated trace amounts in both cardiac and peripheral blood. Also, heart, liver and lung tissue extracts revealed trace concentrations. Generally, all blood and tissue extracts had very close concentrations (Fig. 9).

Interestingly, midazolam concentrations in blood of 15 minutes postmortem at 4°C group (M-PM4) showed an increase in all blood extracts when compared to antemortem control group (M-AM), which was statistically significant for cardiac blood (P<0.05) and non significant for peripheral blood (P>0.05). Likewise, all tissue extracts revealed rise in midazolam concentrations that was statistically significant for heart, liver and lung (P<0.05 each) (Table 3, Fig. 10).

In contrast to M-PM4, midazolam concentrations in 15 minutes postmortem at 21°C group (M-PM21) when compared to antemortem control group (M-AM) illustrated a significant decrease in all blood extracts whether peripheral or cardiac (P<0.05 each). In addition, heart and liver extracts showed significant decreases (P<0.05 each), while lung revealed a significant increase (P<0.05 each) when compared to antemortem control group (M-AM). (Table 4, Fig. 11).

Midazolam peaks were detected in chromatograms of antemortem control M-AM and postmortem groups, M-PM4 and M-PM21 at retention time (RT) = 6.34 – 6.43 (Figs. 12, 13 and 14).

B- Postmortem redistribution markers:

Early phase redistribution in 15 minutes postmortem was evaluated using Cardiac blood to peripheral blood ratio (C/P) and Liver to peripheral blood ratio (L/P) for both lidocaine and midazolam.

1- Cardiac blood to peripheral blood ratio:

Lidocaine postmortem groups at 4°C and 21°C had C/P ratio mean values of 0.878 and 0.799, respectively. Consequently, rats stored at both temperatures gave C/P ratios less than 1, which indicates that no redistribution was detected using this marker for both L-PM4 and L-PM21 groups.

As regard midazolam postmortem groups at 4°C and 21°C, C/P ratio mean values were 1.08 and 0.99, respectively. Accordingly, rats stored at 4°C in M-PM4 group revealed minimal redistribution with C/P ratio just above, but very close to 1. However, rats stored at 21°C in M-PM21 group demonstrated no redistribution with C/P ratio below 1 (Table 5).

2- Liver to peripheral blood ratio:

Lidocaine postmortem groups at 4°C and 21°C had L/P ratio mean values of 0.98 and 31.68, respectively. Consequently, rats stored at 4°C in L-PM4 group revealed no redistribution with L/P ratio below 5. However, rats stored at 21°C in L-PM21 group demonstrated very high redistribution with L/P ratio far above 20.

In case of midazolam postmortem groups at 4°C and 21°C, L/P ratio mean values were 1.03 and 0.99, respectively. Consequently, rats stored at both temperatures gave L/P ratios less than 5, which indicates that no redistribution was detected using this marker for both M-PM4 and M-PM21 groups (Table 6).

Table (1): Statistical comparison between antemortem control (L-AM) and 15 minutes postmortem at 4°C (L-PM4), as regard mean values of lidocaine concentration in peripheral blood, cardiac blood, heart, liver and lung extracts using t test

Groups / Blood/Tissue extracts (ug/ml)	L-AM Mean ± SD N = 6	L-PM4 Mean ± SD N = 6	P value
Peripheral blood	0.0309± 0.0091	0.0457± 0.0146	0.0682
Cardiac blood	0.0284± 0.0099	0.0383± 0.0124	0.1562
Heart	0.1127± 0.0102	0.0535± 0.0139	<0.0001*
Liver	0.0504± 0.0132	0.0409± 0.0074	0.1618
Lung	0.0728± 0.0102	0.0475± 0.0022	0.0019*

N: Number of rats in each group=6 rats SD: standard deviation
P: Probability P: >0.05 non significant P: < 0.05 significant marked with *
L-AM: Lidocaine antemortem control L-PM4: Lidocaine postmortem at 4°C

Table (2): Statistical comparison between antemortem control (L-AM) and 15 minutes postmortem at 21°C (L-PM21), as regard mean values of lidocaine concentration in peripheral blood, cardiac blood, heart, liver and lung extracts using t test

Groups / Blood/Tissue extracts (ug/ml)	L-AM Mean ± SD N = 6	L-PM21 Mean ± SD N = 6	P value
Peripheral blood	0.0309± 0.0091	0.0518± 0.0054	0.0013*
Cardiac blood	0.0284± 0.0099	0.0411± 0.0022	0.0277*
Heart	0.1127± 0.0102	0.0486± 0.0070	<0.0001*
Liver	0.0504± 0.0132	1.6187± 0.2199	<0.0001*
Lung	0.0728± 0.0102	1.4070± 0.3708	0.0003*

N: Number of rats in each group=6 rats SD: standard deviation
P: Probability P: >0.05 non significant P: < 0.05 significant marked with *
L-AM: Lidocaine antemortem control L-PM21: Lidocaine postmortem at 21°C

Table (3): Statistical comparison between antemortem control (M-AM) and 15 minutes postmortem at 4°C (M-PM4), as regard mean values of midazolam concentration in peripheral blood, cardiac blood, heart, liver and lung extracts using t test

Groups / Blood/Tissue extracts (ug/ml)	M-AM N = 6 Mean ± SD	M-PM4 N = 6 Mean ± SD	P value
Peripheral blood	0.5320± 0.0010	0.5327± 0.0035	0.6290
Cardiac blood	0.5299± 0.0013	0.5767± 0.0213	0.0030*
Heart	0.5305 ± 0.0019	0.5384± 0.0012	<0.0001*
Liver	0.5299± 0.0014	0.5494± 0.0041	<0.0001*
Lung	0.5286± 0.0002	0.5305± 0.0017	0.0395*

N: Number of rats in each group=6 rats SD: standard deviation
P: Probability P: >0.05 non significant P: < 0.05 significant marked with *
M-AM: Midazolam antemortem control M-PM4: Midazolam postmortem at 4°C

Table (4): Statistical comparison between antemortem control (M-AM) and 15 minutes postmortem at 21°C (M-PM21), as regard mean values of midazolam concentration in peripheral blood, cardiac blood, heart, liver and lung extracts using t test

Groups / Blood/Tissue extracts (ug/ml)	M-AM N = 6 Mean ± SD	M-PM21 N = 6 Mean ± SD	P value
Peripheral blood	0.5320± 0.0010	0.5286± 0.0004	0.0001*
Cardiac blood	0.5299± 0.0013	0.5283± 0.0000	0.0279*
Heart	0.5305 ± 0.0019	0.5284± 0.0002	0.0376*
Liver	0.5299± 0.0014	0.5283± 0.0000	0.0414*
Lung	0.5286± 0.0002	0.5292± 0.0005	0.0186*

N: Number of rats in each group=6 rats SD: standard deviation
P: Probability P: >0.05 non significant P: < 0.05 significant marked with *
M-AM: Midazolam antemortem control M-PM21: Midazolam postmortem at 21°C

Table (5): Cardiac blood to peripheral blood ratio (C/P) illustrates the degree of Lidocaine and Midazolam redistribution 15 minutes postmortem at 4°C and 21°C

Group / Drug	C/P ratio	
	PM4	PM21
	N = 6 Mean ± SD	N = 6 Mean ± SD
Lidocaine	0.878568± 0.2935	0.799015± 0.0754
Midazolam	1.082436± 0.0378*	0.999497± 0.0008

N: Number of rats in each group=6 rats SD: standard deviation
PM4: 15 minutes postmortem at 4°C PM21: 15 minutes postmortem at at 21°C
C/P: Cardiac blood to peripheral blood ratio, if > 1 postmortem redistribution marked with *

Table (6): Liver to peripheral blood ratio (L/P) illustrates the degree of Lidocaine and Midazolam redistribution 15 minutes postmortem at 4°C and 21°C

Group / Drug	L/P ratio	
	PM4	PM21
	N = 6 Mean ± SD	N = 6 Mean ± SD
Lidocaine	0.981265± 0.3685	31.68046± 6.2579*
Midazolam	1.031338± 0.0130	0.999484± 0.0008

N: Number of rats in each group=6 rats SD: standard deviation
PM4: 15 minutes postmortem at 4°C PM21: 15 minutes postmortem at at 21°C
L/P: Liver to peripheral blood ratio, if > 20 postmortem redistribution marked with *

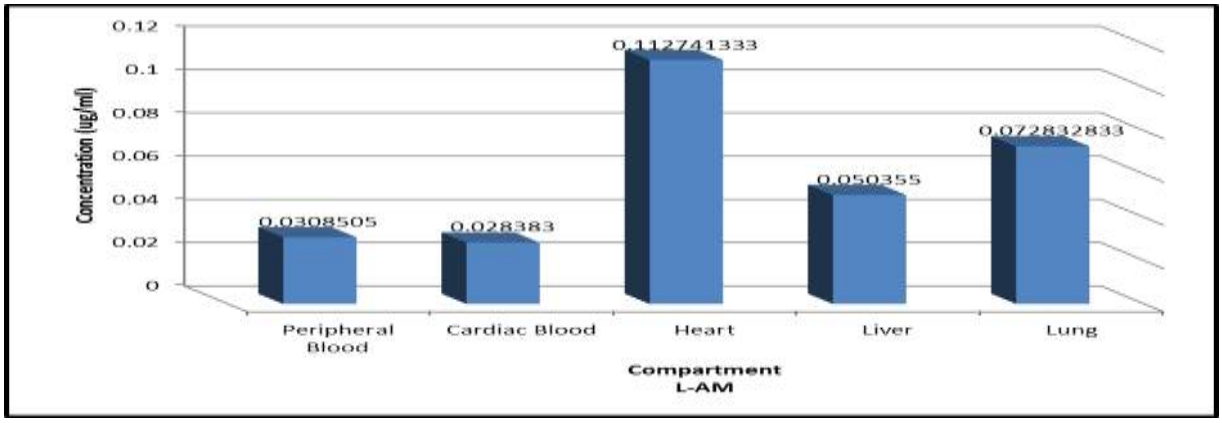

Fig. (3): Bar chart showing mean values of lidocaine distribution in rats after subcutaneous dosage, 30 minutes between application and scarification, in lidocaine antemortem control group L-AM

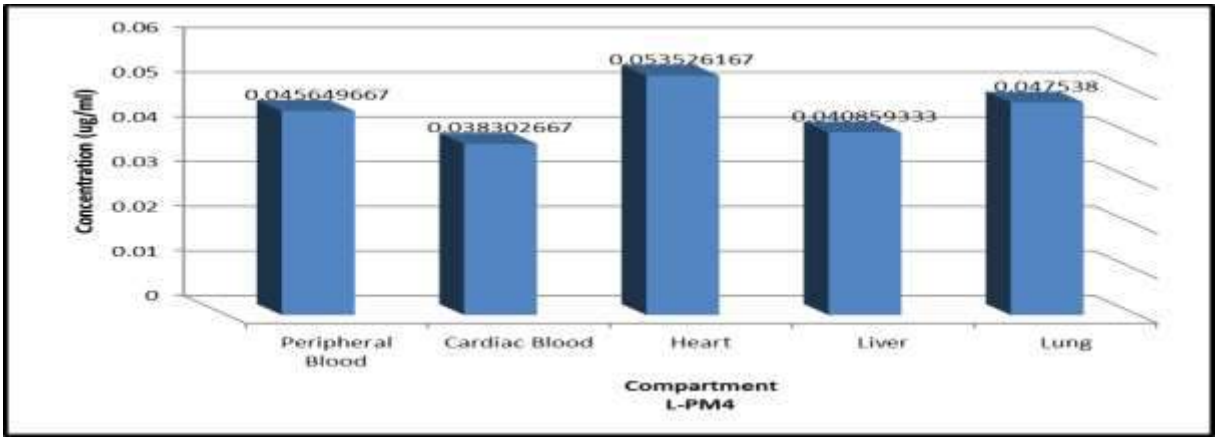

Fig. (4): Bar chart showing mean values of lidocaine distribution in rats after subcutaneous dosage, 30 minutes between application and scarification, and rats were left in supine position for 15 minutes at 4°C L-PM4

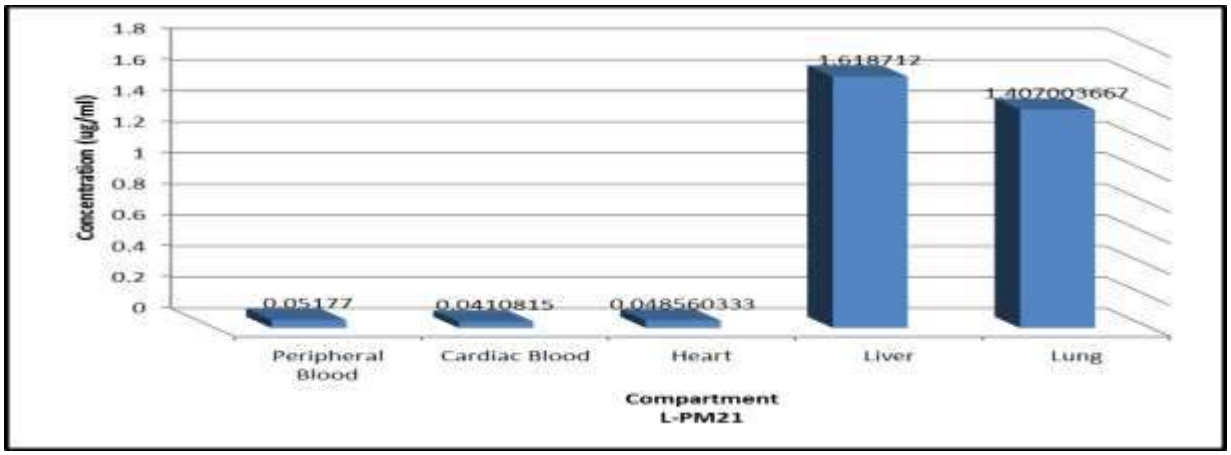

Fig. (5): Bar chart showing mean values of lidocaine distribution in rats after subcutaneous dosage and 30 minutes between application and scarification then left in supine position for 15 minutes at 21°C in postmortem lidocaine group L-PM21

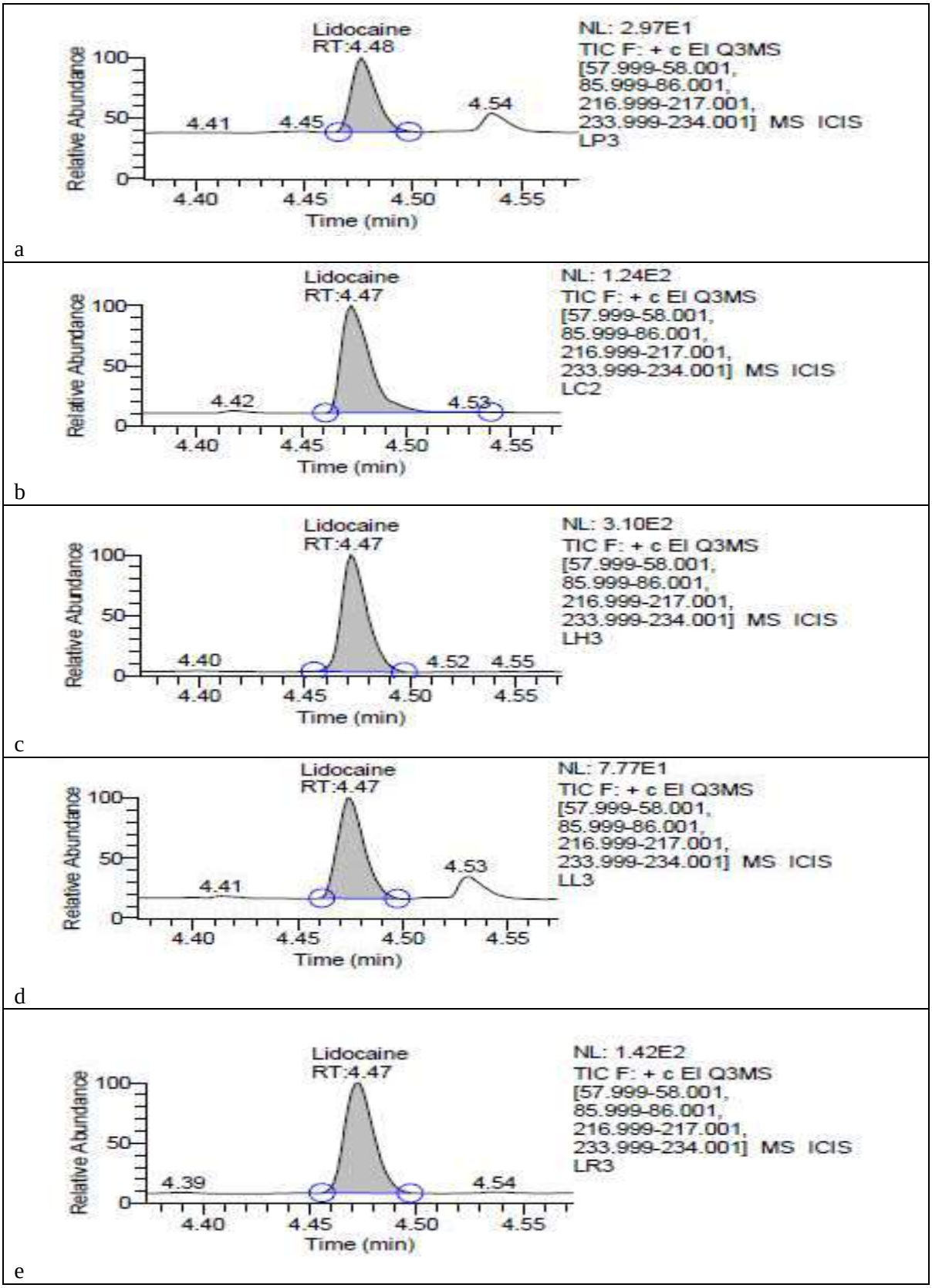

Fig. (6): Chromatogram showing lidocaine peaks in rats after subcutaneous dosage and 30 minutes between application and scarification, in antemortem lidocaine control group L-AM: a) peripheral blood, b) cardiac blood, c) heart, d) liver, e) lung

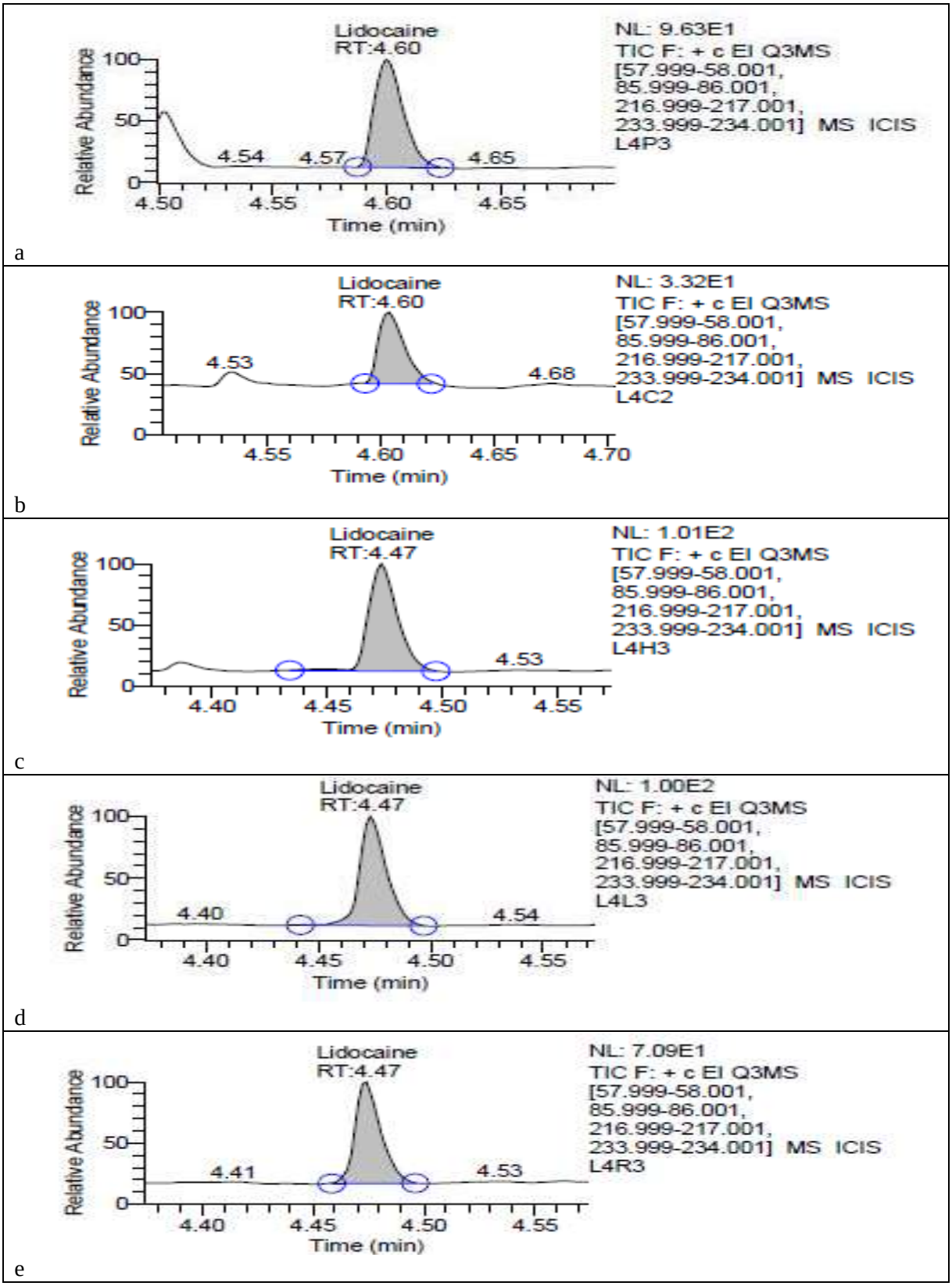

Fig. (7): Chromatogram showing lidocaine peaks in rats after subcutaneous dosage and 30 minutes between application and scarification, then left in supine position for 15 minutes at 4°C in postmortem lidocaine group L-PM4: a) peripheral blood, b) cardiac blood, c) heart, d) liver, e) lung

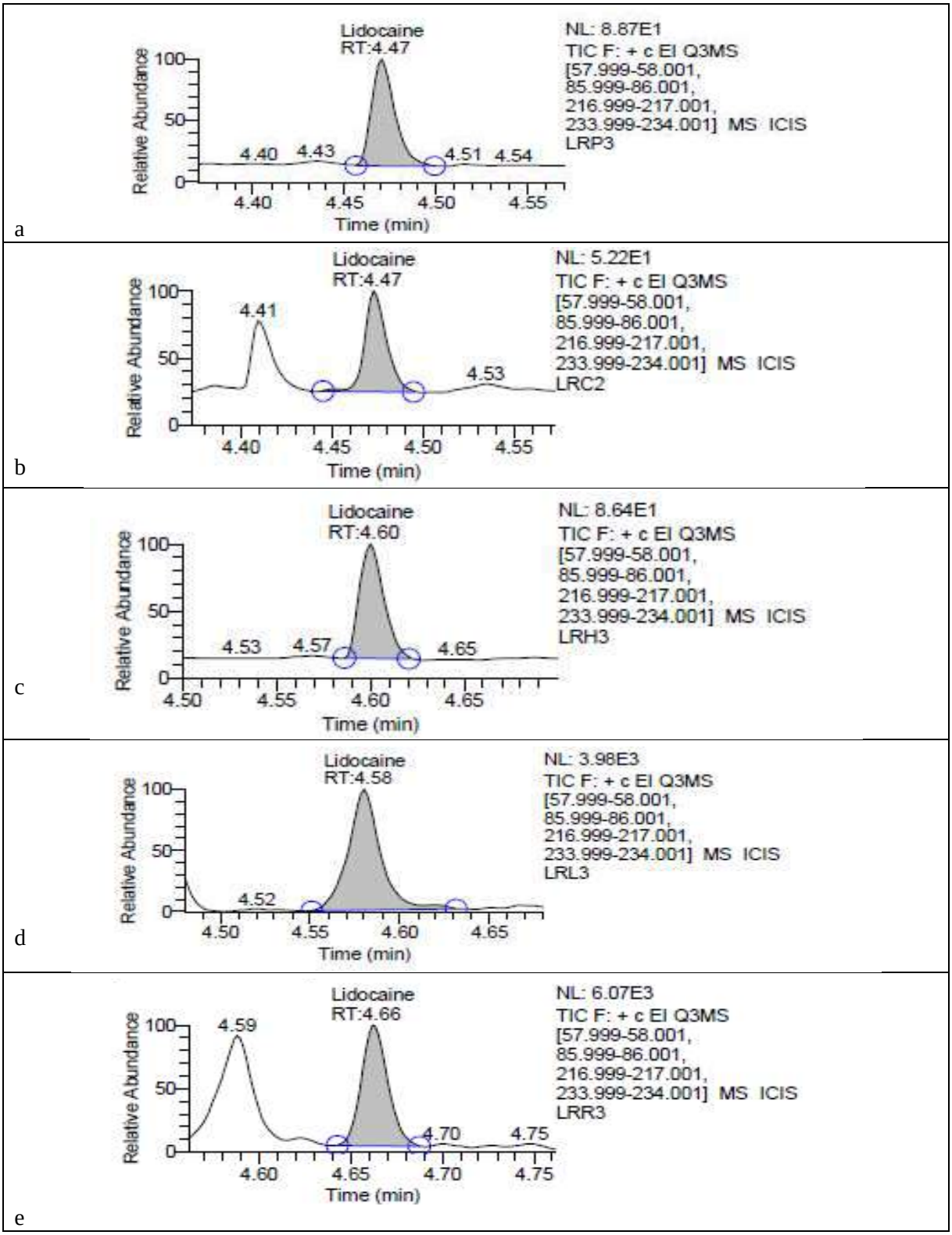

Fig. (8): Chromatogram showing lidocaine peaks in rats after subcutaneous dosage and 30 minutes between application and scarification, then left in supine position for 15 minutes at 21°C in postmortem lidocaine group L-PM21: a) peripheral blood, b) cardiac blood, c) heart, d) liver, e) lung

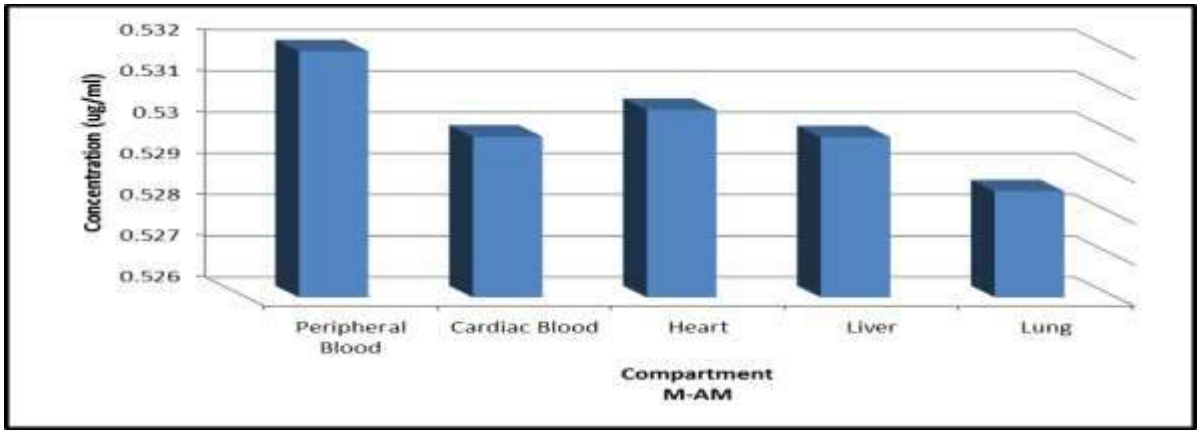

Fig. (9): Bar chart showing mean values of midazolam distribution in rats after intravenous dosage, 30 minutes between application and scarification, in midazolam antemortem control group M-AM

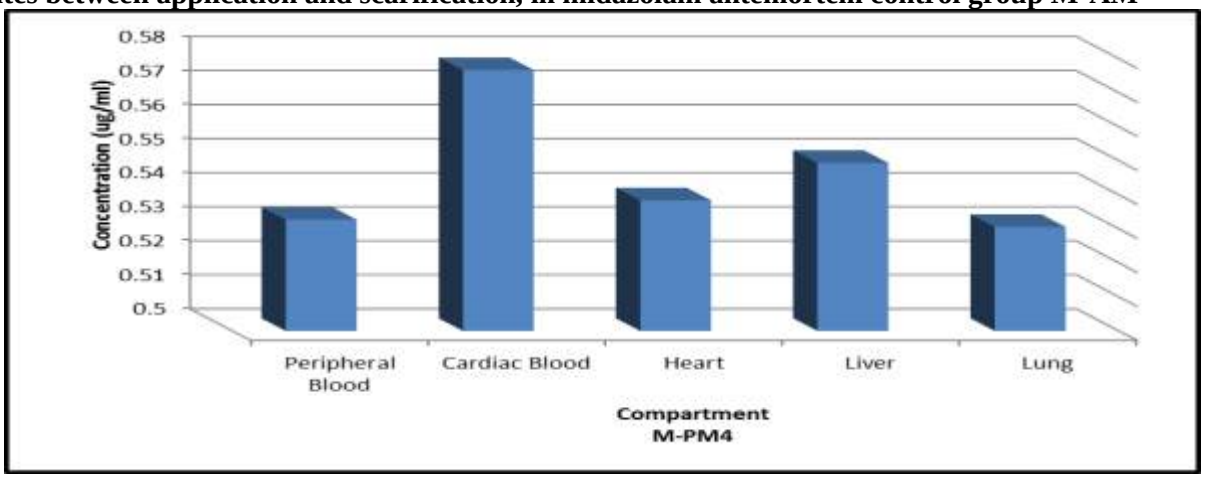

Fig. (10): Bar chart showing mean values of midazolam distribution in rats after intravenous dosage, 30 minutes between application and scarification, and rats were left in supine position for 15 minutes at 4°C M-PM4

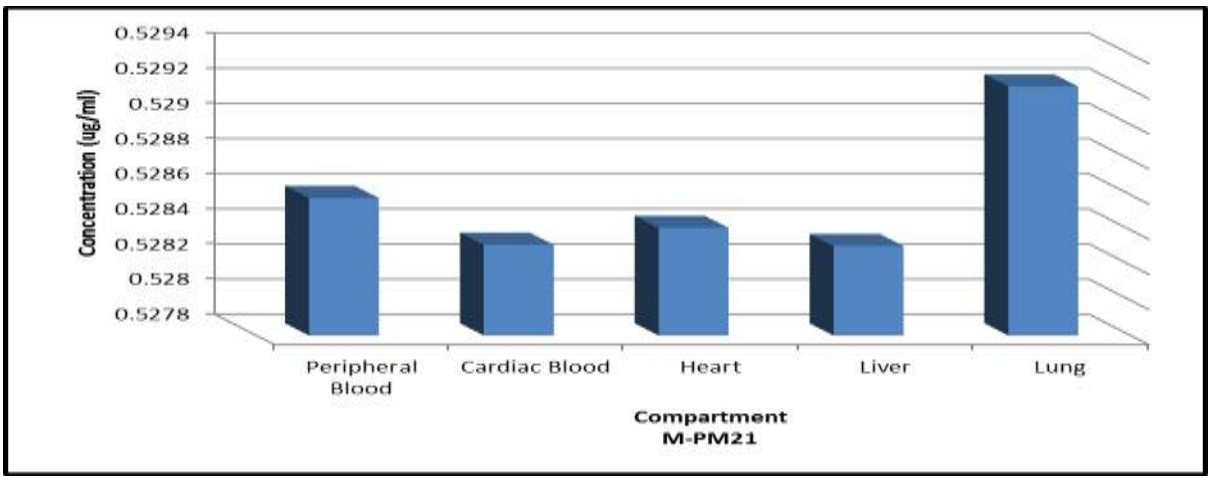

Fig. (11): Bar chart showing mean values of midazolam distribution in rats after intravenous dosage, 30 minutes between application and scarification, and rats were left in supine position for 15 minutes at 21°C M-PM21

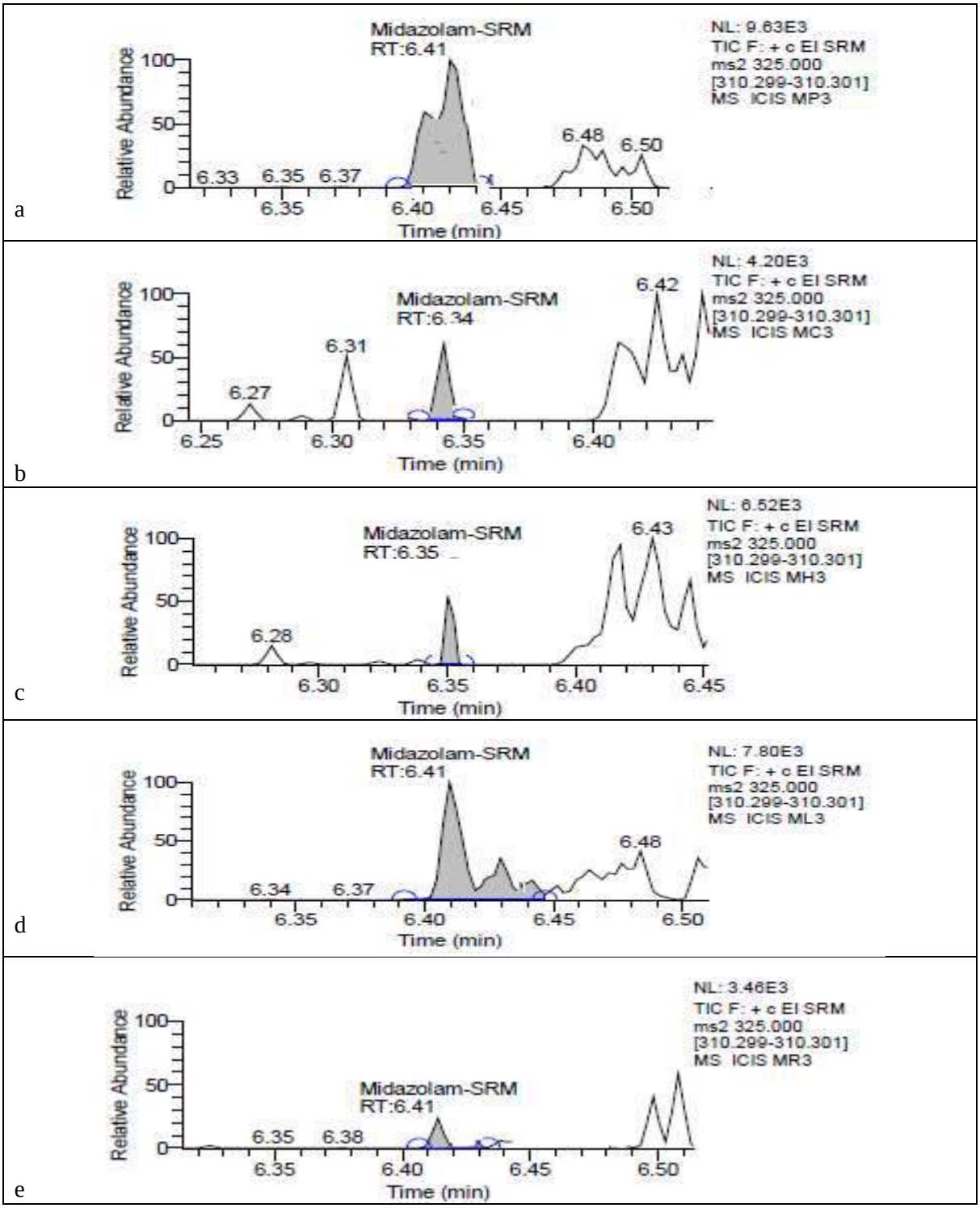

Fig. (12): Chromatogram showing midazolam peaks in rats after intravenous dosage and 30 minutes between application and scarification, in antemortem midazolam control group M-AM: a) peripheral blood, b) cardiac blood, c) heart, d) liver, e) lung

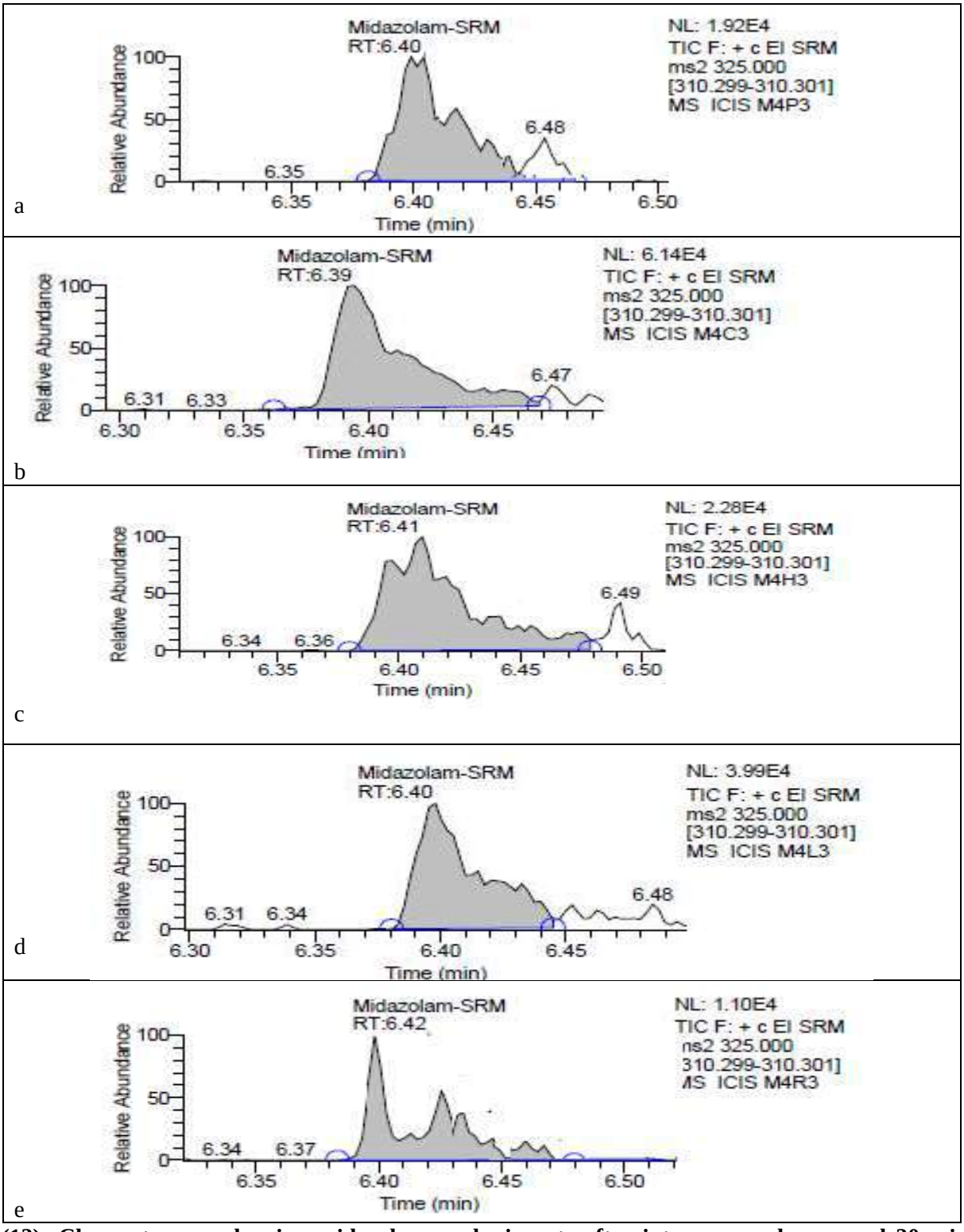

Fig. (13): Chromatogram showing midazolam peaks in rats after intravenous dosage and 30 minutes between application and scarification, then left in supine position for 15 minutes at 4°C in postmortem midazolam group M-PM4: a) peripheral blood, b) cardiac blood, c) heart, d) liver, e) lung

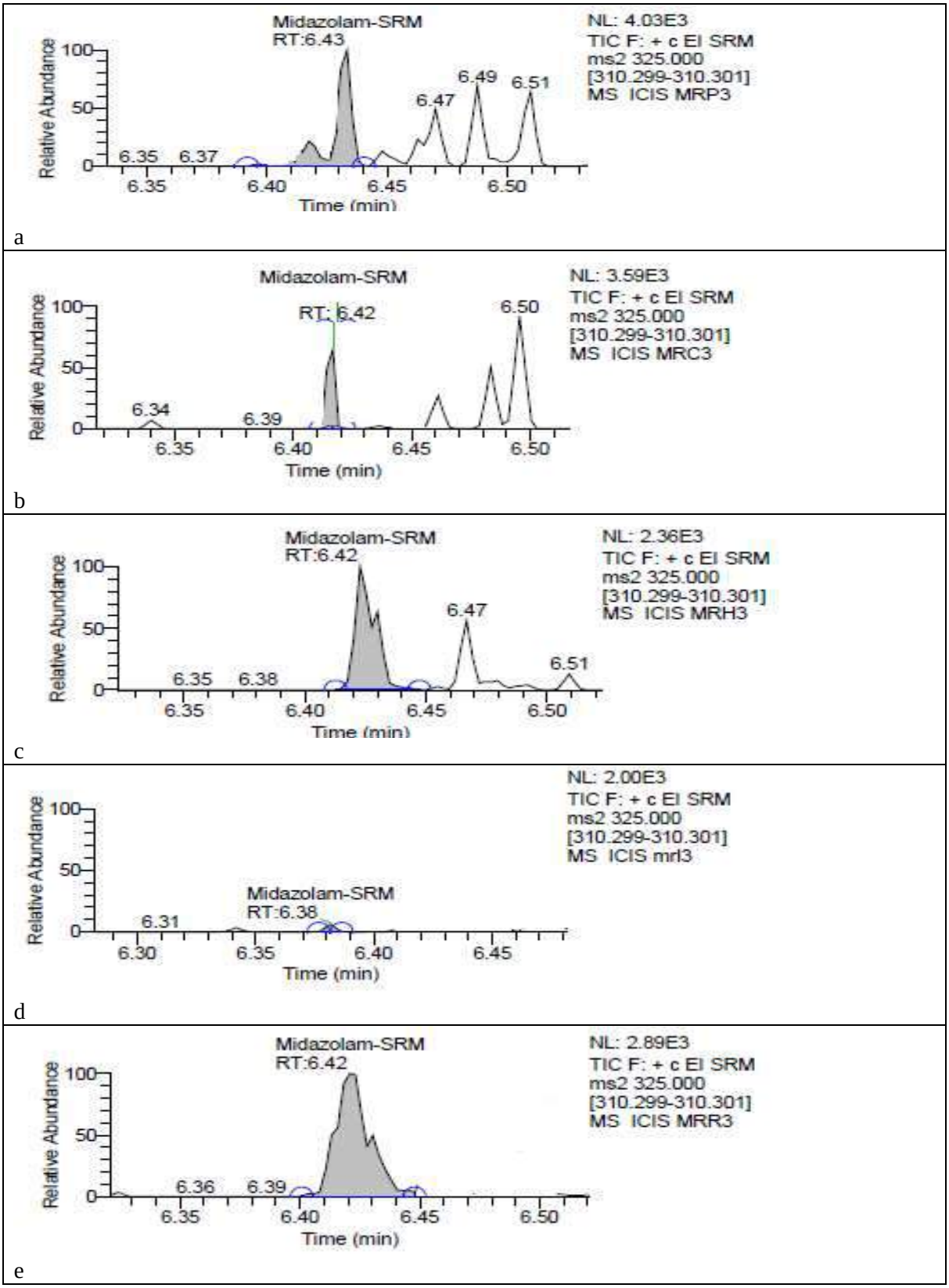

Fig. (14): Chromatogram showing midazolam peaks in rats after intravenous dosage and 30 minutes between application and scarification, then left in supine position for 15 minutes at 21°C in postmortem midazolam group M-PM21: a) peripheral blood, b) cardiac blood, c) heart, d) liver, e) lung

DISCUSSION

The current study revealed that lidocaine accumulated in tissues rather than blood (myocardium, lung and liver, respectively). Moreover, its concentration in peripheral blood was slightly higher than cardiac blood in all studied groups. Lidocaine concentration in postmortem group stored at 4ºC revealed non-significant increase in blood (cardiac and peripheral), while tissues revealed significant decline in heart and lung and non-significant decline in the liver when compared to antemortem control group. On the other hand, lidocaine concentration in postmortem group kept at 21ºC showed significant increase in blood concentration (cardiac and peripheral) and significant decrease in heart concentration as expected. Surprisingly, liver and lung revealed significant increase in lidocaine concentration when compared to antemortem control group.

McIntyre, 2016 stated that basic lipophilic drugs with volume of distribution greater than 3 L/kg are particularly more prone to PMR. This was in line with our results, the physicochemical properties of lidocaine suggest its strong potential for redistribution (weak lipophilic base, high protein binding 60-80%, pKa 7.9 and volume of distribution 1.1 to 2.1 L/kg) **(Estebe, 2017** and **Wei et al., 2017)**.

The pKa is referred to the pH at which the ratio of unprotonated to protonated drug is unity. The effect of pH alterations on the state of an ionizable drug can be deduced from its pKa. If the protonated state of a drug is ionized, then the more acidic the medium, the more ionized drug will be present; and vice versa. A number of factors affect the volume of distribution such as the lipid solubility of the drug and the extent of its binding to plasma protein. Xenobiotics that are highly bound to plasma proteins would be expected to have a low volume of distribution equal to that of the plasma volume; and vice versa **(Ferner, 2008)**.

Wasson et al., 2019 stated that lidocaine is a weak base as the tertiary amine acts as a proton acceptor and the unionized form is lipid soluble and diffuses through the cell membrane.

In addition, **Yarema and Becker, 2005** explained the liability of basic drugs to distribute more readily postmortem by the increasing intracellular acidity of cell contents that are highly aqueous. Thus, a basic drug will be progressively more ionized intracellular, then after cell lysis it will distribute more easily (i.e. being transported in the acidic fluid in which they are dissolved).

Oertel et al., 2015 studied the distribution of lidocaine in 51 human cases in in relation to the time between lidocaine application and death. Most of IV lidocaine was found in cardiac blood after a short time of distribution. Longer time revealed that the highest levels were in brain. Finally, in the excretion phase the highest concentration was found in kidney and in urine. Equally distributed lidocaine was found in >20 cases indicated its usage as a local anesthetic. They stated that similar lidocaine levels in cardiac and peripheral blood was expected in patients with continuous dosage or local application with gradual distribution. However, in IV cases with rapid sampling, higher lidocaine levels were in cardiac blood than venous blood due to its incomplete distribution after injection. In 7 cases peripheral vein revealed higher concentrations than in cardiac blood and they suggested that lidocaine was normally distributed only a short time after injection, and then the blood circulated only in the trunk. Also, the elimination process occured only in this central area and the remaining lidocaine stayed in the peripheral areas with higher concentrations measured in the femoral vein.

McIntyre, 2016 stated that in cases of acute toxicities the incomplete distribution of xenobiotics can result in variable levels throughout the tissues and organs of the body. Also, **Leikin and Watson, 2003** recommended the brain, lung, heart and liver as tissue sites to determine acute fatal lidocaine toxicity.

Moreover, **Estebe, 2017** documented that the main targets of lidocaine are brain and heart. He also attributed lung trapping of lidocaine to the lower pulmonary pH than plasma. Redistribution from brain could partly explain

our result in 21ºC postmortem group that revealed marked increase in lung and liver concentrations that couldn't be explained by the decline in cardiac concentration alone.

The present study revealed that C/P ratio in 15 minutes postmortem groups (at 4ºC and 21ºC) were less than 1; indicating that early phase PMR wasn't detected in rats stored at both temperatures by using this marker. In contrast, L/P ratio revealed that PMR was arrested in rats stored at 4ºC with ratios less than 5, while rats kept at 21ºC revealed high degree of PMR with ratios above 20. This suggests that lidocaine was prone to early phase PMR, however cold storage environment could significantly arrest this process by using L/P ratio.

Therefore, L/P ratio was a more indicative marker of this early redistribution than C/P ratio that couldn't detect any PMR in both postmortem groups. This could be a result of the slightly higher lidocaine concentration in peripheral blood than cardiac blood in all our studied groups. According to **Oertel et al., 2015,** this could be explained by the local route of lidocaine administration (SC administration in the lower abdominal region). In addition, **Gerostamoulos et al., 2012** postulated that potential sources for redistributed drugs in peripheral samples include the bladder, skeletal muscle, and body fat.

Several studies were consistent with our results and proved that lidocaine was liable to undergo PMR via C/P ratios more than 1 **(Leikin and Watson, 2003; Han et al., 2012** and **Wei et al., 2017**). Howerver, few literatures are available that compare PMR of drugs at different temperatures.

Kugelberg et al., 2005 studied the influence of storage temperature (4ºC versus 21 ºC**)** on early phase PMR (15 minutes) of the antidepressant citalopram in rats. Citalopram was administered subcutaneously to exclude any functional drug depot present in the gastrointestinal tract. They found that refrigeration at 4ºC did not prevent, but significantly reduced PMR as compared to rats stored at 21 ºC. In addition, they attributed the early change in postmortem concentrations within 15 minutes to the small sized animal model they used (rats). They also explained the passive diffusion from drug depots in solid organs particularly lungs to cardiac blood by the basic lipophilic nature of drugs such as citalopram that increase their tendency to accumulate in pulmonary cells, in addition to the thin diffusion membrane, large surface area of the alveoli, and high lung vascularization.

Pélissier-Alicot et al., 2003 stated that PMR is influenced by physical factors such as the temperature of the corpse, time between death and sampling (i.e. refrigeration at 4ºC hinders the PMR and the delay between death and autopsy increases it).

A previous study of **Pounder and Smith**, **1995** investigated the postmortem ethanol diffusion from the stomach in humans. They reported that PMR was markedly suppressed by refrigeration at 4ºC.

Regarding the midazolam group, the present work showed that midazolam existed in trace amounts with nearly equal concentrations in blood and tissue extracts of all studied groups. In postmortem group stored at 4ºC, midazolam revealed significant increase in cardiac blood concentration and non-significant increase in peripheral blood; in addition it revealed significant increase in all tissue extracts (heart, lung and liver) when compared to antemortem control group. On the other hand, midazolam in postmortem group kept at 21ºC showed significant decline in all blood and tissue concentrations except lung that revealed significant increase when compared to antemortem control group.

In line with current literature, the physicochemical properties of midazolam suggests its weak potential for redistribution (lipophilic, high protein binding 96%, pKa 6.2 and volume of distribution 0.7-1.7 L/kg) **(Olkkola and Ahonen, 2008** and **Riss et al., 2008)**.

According to **Ferner, 2008,** drugs having nonuniform distribution before death, their postmortem distribution will be more uniform due to the flow down concentration gradient from high to lower concentration. Also, there is no further renewal of energy and the integrity

of cell membranes is lost, consequently, cell membranes become freely permeable. Moreover, in cases of highly protein-bound xenobiotics (e.g. midazolam) the plasma proteins act as a reservoir of inactive, but circulating, fraction of the drug. Distribution of protein between the blood and interstitial fluid relies on the maintenance of a semipermeable membrane. Therefore, alterations in membrane permeability postmortem allow proteins to leak out of the bloodstream into tissues, lowering the concentration of albumin-bound drug in blood.

According to **Skopp, 2010,** midazolam undergoes degradation rapidly under postmortem acidic conditions and drug degradation during the postmortem interval competes with PMR. Thus, it was not surprising to observe in our results that midazolam concentrations decreased substantially at 21ºC postmortem. This wasn't the case in rats kept at 4 ºC, may be due to the arrest of metabolic activities and the postmortem changes that reduce the pH after death.

In addition, **Pélissier-Alicot et al., 2003** postulated that some compounds such as benzodiazepines and ethanol could be metabolized by microorganisms during the putrefactive process.

Gerostamoulos et al., 2012 stated that environmental conditions, trauma, and putrefaction can significantly influence the degree of alteration in postmortem drug concentrations. Accordingly, they explained their results on autopsy cases that revealed postmortem reduction of amphetamine and methamphetamine and were inconsistent with previous literatures that suggested their increase after death.

According to **Griffin III et al., 2013** midazolam is one of the most lipophilic benzodiazepines and highly concentrated in lipid-rich areas (e.g. the central nervous system and adipose tissue). This could explain the unexpected rise of midazolam concentrations in blood and tissue extracts that took place at 4 ºC by redistribution from areas of high concentrations. This wasn't the case in rats kept at 21 ºC that showed significant reduction in midazolam concentrations most probably due to its degradation in the progressive acidic medium.

The present study showed that C/P ratio of midazolam 15 minutes postmortem group at 4ºC was just above 1; indicating minimal early phase PMR, while rats kept at 21ºC gave ratios just below 1 denoting no PMR using this marker. By using L/P ratio, midazolam 15 minutes postmortem groups (at 4ºC and 21ºC) had ratios less than 5; indicating that early phase PMR wasn't detected in rats stored at both temperatures by using this marker. These results were suggestive that midazolam was not prone to early phase PMR at both storage temperatures by L/P ratio as a marker. This was in harmony with C/P ratio for rats stored at 21ºC, however rats stored in cold environment revealed minimal PMR by using C/P ratio as a marker.

While it has been expected from previous studies that midazolam reveals redistribution with C/P ratio 4 (**Ferner, 2008** and **Skopp, 2010**). This wasn't apparent in our results except for rats stored at 4ºC with mean C/P ratio 1.08 that revealed minimal redistribution. However, L/P ratio for all our studied groups revealed no redistribution this could be a result of early measurement, 15 minutes postmortem, as well as the presence of midazolam in trace amounts most probably due to degradation.

According to **Rowshan, 2014** midazolam, as a benzodiazepine, undergoes very little PMR because it is not highly concentrated in the major organs relative to blood.

In harmony with our results **Leikin and Watson, 2003; Han et al., 2012** and **Wei et al., 2017** revealed that midazolam wasn't liable to undergo PMR with C/P ratios less than 1 and L/P ratios less than 5.

To the best of our knowledge, no published literature are available that compare PMR of midazolam at different storage temperatures. However, **Yarema and Becker, 2005** reported that the nitrobenzodiazepines (clonazepam, nitrazepam, and flunitrazepam) are biotransformed by enterobacteria found in the

gastrointestinal tract after death. The rate of this bioconversion is slowed down by refrigeration of the corpses at 4ºC compared to storing at temperatures ranging from 22 to 37 ºC. This emphasizes the importance of preservation of corpses at cool temperatures.

CONCLUSIONS

L/P ratio was a more reliable marker for PMR than C/P ratio. By using L/P ratio, lidocaine revealed no PMR at 4ºC and great liability for PMR at 21 ºC. However, midazolam revealed no PMR at both temperatures. On the other hand, C/P ratio revealed that lidocaine didn't undergo any PMR at both temperatures. However, midazolam revealed minimal PMR at 4ºC and no PMR at 21 ºC.

RECOMMENDATIONS

On the light of the results of the present study, the following guidelines are recommended:

1-In interpretation of postmortem lidocaine and midazolam concentrations, the implication of PMR of both drugs and postmortem degradation of midazolam should be considered.

2- Postmortem blood samples should be sampled from peripheral vessels at the time of mortuary admission and cadavers should be kept at 4ºC.

3- Moreover, L/P ratio as a marker of PMR was more reliable than C/P ratio.

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المرحلة المبكرة لإعادة توزيع ما بعد الوفاه لليدوكين و الميدازولام فى الجرذان البيضاء البالغة غير

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مقدمة البحث: إعادة التوزيع بعد الوفاة هي التغيرات التي تحدث في تركيزات الدواء بعد الموت، ويعد الليدوكين هو المخدر الموضعي الأكثر شيوعًا في جميع أنحاء العالم ، أما الميدازولام فهو مزيل للقلق ومهدئ يستخدم قبل التخدير.
هدف البحث: الهدف من هذا البحث هو دراسة المرحلة المبكرة لإعادة الإنتشار بعد الوفاة لكلا من الليدوكين و الميدازولام، ودراسة تأثير درجة حرارة التخزين (4 و21 درجة مئوية) على إعادة انتشارهم في ذكور الجرذان البيضاء البالغة. وذلك وذلك بإجراء ما يلى: قياس تركيزاتهم فى الدم (الدم المركزى من القلب و الدم المحيطي من الوريد الحرقفي الخارجي) و الأنسجة (القلب والرئتين والكبد) بواسطة جهاز كروماتوغرافيا الغاز - مطياف الكتلة (GC-MS)، و حساب نسبة التركيز فى دم القلب إلى التركيز فى الدم المحيطى (C / P)، ونسبة التركيز فى الكبد إلى التركيز فى الدم المحيطى (L / P).
طريقة البحث: اجرى البحث على 36 من الجرذان البيضاء البالغة من الذكور، تم تقسيمهم إلى مجموعتين رئيسيتين- الليدوكين و الميدازولام - (18 جرذا في كلا منهما). **المجموعة الأولى (الليدوكين):** تم حقن الجرذان تحت الجلد بهيدروكلوريد الليدوكين 2% (67 مجم/ كجم)، و بعد مرور 30 دقيقة من الحقن تمت التضحية بالجرذان. و قد تم تقسيمهم إلى 3 مجموعات فرعية متساوية كما يلى: **ل-ق** (مجموعة الضابطة قبل الوفاة)، **ل-ب4** (تم وضع الجرذان على ظهورها لمدة 15 دقيقة بعد الوفاة عند 4 درجة مئوية)، **ل-ب21** (تم وضع الجرذان على ظهورها لمدة 15 دقيقة بعد الوفاة عند21 درجة). **المجموعة الثانية (الميدازولام):** تم حقن الجرذان فى الوريد بالميدازولام (75 مجم/ كجم)، و بعد مرور 30 دقيقة من الحقن تمت التضحية بالجرذان و قد تم تقسيمهم إلى 3 مجموعات فرعية متساوية كما يلى: **م-ق** (مجموعة الضابطة قبل الوفاة)
م-ب4(تم وضع الجرذان على ظهورها لمدة 15 دقيقة بعد الوفاة عند 4 درجة مئوية).
النتائج: كانت هناك تغييرات كبيرة في تركيزات الليدوكين والميدازولام في كل من الأنسجة وعينات الدم مقارنة مع لمجموعات قبل الوفاة الضابطة المقابلة. و أظهرت دلالات إعادة الإنتشار بعد الوفاة أن نسبة تركيز الليدوكين فى دم القلب إلى تركيزه فى الدم المحيطى (C/P) في مجموعات ل-ب4 و ل-ب21 كانت أقل من 1 ؛ مما يشير إلى أن المرحلة الأولى من إعادة الإنتشار بعد الوفاة لم يتم ملاحظتها في الجرذان المحفوظة في كلتا الحرارتين باستخدام هذه الدلالة. في المقابل ، كشفت نسبة تركيز الليدوكين فى الكبد إلى تركيزه فى الدم المحيطى (L/P) على أنه قد تم تثبيط المرحلة الأولى من إعادة الإنتشار بعد الوفاة في جرذان المجموعة ل-ب4 بنسب أقل من 5 ، في حين أظهرت جرذان المجموعة ل-ب21 درجة عالية من إعادة الإنتشار بعد الوفاة. مع نسب أعلى من 20. وهذا يدل على أن الليدوكين كان عرضة لإعادة الإنتشار بعد الوفاة ، ولكن درجة حرارة التخزين البارد أعاقت بشكل كبير هذه العملية باستخدام نسبة L/P كدلالة. كما أظهرت الدراسة الحالية أن نسبة تركيز الميدازولام فى دم القلب إلى تركيزه فى الدم المحيطى (C/P) في مجموعة م-ب4 كانت أعلى من 1 بقليل؛ مما يدل على حدوث حد أدنى من المرحلة الأولى من إعادة الإنتشار بعد الوفاة، في حين أعطت مجموعة ل-ب21 نسب أقل من 1 مما يشير إلى أن المرحلة الأولى من إعادة الإنتشار بعد الوفاة لم يتم ملاحظتها في جرذان هذه المجموعة باستخدام هذه الدلالة. باستخدام نسبة تركيز الميدازولام فى الكبد إلى تركيزه فى الدم المحيطى (L/P)، كان لكلا من المجموعتين م-ب4 و م-ب21 نسب أقل من 5 ؛ مما يشير إلى أن الميدازولام لم يكن عرضة لإعادة الإنتشار بعد الوفاة في الجرذان المخزنة في كلتا الحرارتين باستخدام هذه الدلالة.
الخلاصة: وجد أن الليدوكين كان عرضة للمرحلة الأولى من إعادة الإنتشار بعد الوفاة كما هو مبين بنسب L/P تخطت 20 عند 21 درجة مئوية، كما أن درجة حرارة 4 مئوية اثبطت إعادة انتشاره بعد الوفاة كما هو مبين بنسب C/P أقل من 1 و نسب L/P أقل من 5.أما الميدازولام فقد تعرض للفقد بعد الوفاة و كان عرضة بدرجة بسيطة للمرحلة الأولى من إعادة الإنتشار بعد الوفاة كما هو مبين بنسب C/P أعلى قليلا من 1 عند 4 درجة مئوية.
التوصيات: فى ضوء نتائج هذه الدراسة نوصى بالآتى:
1- وضع تأثير ظاهرة إعادة الإنتشار فى الاعتبار عند تفسير التحليل السمي لتركيزات الليدوكين و والميدازولام بعد الوفاة، بالإضافة إلى وضع احتمالية حدوث فقد للميدازولام.
2- أخذ عينات الدم من الأوعية المحيطة (الوريد الفخذي أو الحرقفي الخارجي) في أول وقت دخول الجثث إلى المشرحة، بالإضافة إلى تبريد الجثث بحفظها عند درجة حرارة 4 مئوية.
3- تعتبر نسبة تركيز الأدوية فى الكبد إلى تركيزها فى الدم المحيطى (L/P) أكثر موثوقية من نسبة تركيز الأدوية فى دم القلب إلى تركيزها فى الدم المحيطى (C/P) كمؤشر على حدوث إعادة الإنتشار بعد الوفاة.