Journal of Current Pharma Research (JCPR)
Volume 22, Issue 4, April 2026 **jcpr.humanjournals.com** ISSN: 2230-7842, 2230-7834

Quantitative Analysis of Anti-Depressant Drug Concentration in Bone Marrow by Using HPLC-DAD

N. Priyadharshini*, S. Deva Bharathi

Department of forensic science and criminology, Annai Fathima college of arts and science, Thirumangalam, Madurai, Tamil Nadu, INDIA.

Received: 25 February 2026 Revised: 10 March 2026 Accepted: 27 March 2026

ABSTRACT

In forensic toxicology, the analysis of drugs in alternative biological matrices has grown in significance, particularly when traditional samples like blood and urine are either unavailable or inappropriate. Using HPLC-DAD (High-Performance Liquid Chromatography with Diode-Array Detection), this study examined the amount and stability of tricyclic antidepressants (TCAs), namely amitriptyline and imipramine, within a bone marrow matrix. The target medications were added to bone marrow samples, and the samples were allowed to equilibrate for up to five days. Three separate intervals were used to analyse the samples: 1 hour on Day 0, 72 hours on Day 3, and 120 hours on Day 5. The parent drugs were successfully recovered at a concentration of 0.075 µg/mL in the first analysis at one hour, resulting in a 0.15% recovery rate. Because of their structural similarities, amitriptyline, and imipramine co-eluted at a retention time of 5.787 minutes; The 0th day results verified that the drugs had been successfully extracted from the biological matrix (bone marrow). But a very small amount of the drug was recovered. The parent drug peak at 5.787 minutes vanished after 72 hours of analysis, and a new peak at 5.840 minutes took its place. This implies a reduction or degradation of parent drugs. The parent drugs were completely undetectable by 120 hours, making quantification impossible. The findings show that although bone marrow is a good source for toxicological extraction, amitriptyline and imipramine degrade quickly in this research.

Keywords: HPLC-DAD, Amitriptyline, Imipramine, Bone Marrow, Drug Degradation, Tricyclic Antidepressants.

INTRODUCTION

The objective of this study was to determine the suitability of bone marrow for detecting and measuring the antidepressant drug amount in cases where conventional specimens such as blood or urine are unavailable, decomposed, or unsuitable for analysis. The study also aimed to analyse the quantity and concentration of antidepressant drugs in bone marrow using High- Performance Liquid Chromatography-Diode Array Detector.

Giordano. G (2025)- The study explains how to detect drugs and toxic substances in bone tissue by improving extraction methods and examining the effects of post-mortem changes when usual samples are not available.

Dalsasso. L. C. F & Marchioni. C (2024)- The study examined various research papers on detecting cocaine in post-mortem cases, compared sample types and methods used, and identified common approaches, while noting that more research is still needed.

Kumar. S et al., (2023)- The study reviewed different research on detecting antidepressant drugs in real cases, compared methods used on various samples, highlighted their importance in overdose situations, and pointed out the need for more accurate and sensitive techniques.

Soares. S et al., (2021)- The study brings together existing research on detecting and measuring antidepressant drugs in various biological samples, compares the methods used, and highlights their importance in clinical and forensic settings.

Chang. Y. Y & et al., (2021)-The study analysed ten antitumor drugs in plasma and urine using HPLC-DAD, employed advanced data analysis methods to handle overlapping peaks, and demonstrated accurate detection of multiple drugs in complex biological samples.

Bone marrow is the part of the body that produces blood cells, making up about 3-5% of body weight [1]. Pigs are widely used in biomedical and toxicology research because their bones, bone marrow, and drug distribution closely resemble those of humans, making them a suitable alternative for human studies [2]. Pigs are very similar to humans in body size, structure, immune system, genetics, and physiology, so they are often preferred translational research [3]. Pigs have thigh bones that are very similar to humans in size, structure, healing ability, and mineral content [4].

Antidepressants are used to treat depression, anxiety, and other mental health issues, which are becoming increasingly common [5]. There are many different types of antidepressants available today, such as MAOIs (monoamine oxidase inhibitors), TCAs (tricyclic antidepressants), SSRIs (selective serotonin reuptake inhibitors), and SNRIs (serotonin-norepinephrine reuptake inhibitors) [6]. Tricyclic antidepressants (TCAs) were first introduced in 1959 to treat depression, starting with imipramine, which was originally developed as an antipsychotic and later led to other drugs like amitriptyline, nortriptyline, desipramine, and doxepin [7]. TCAs are usually taken as tablets, capsules, or liquid by mouth, while IV use has been tested in studies but is not commonly used in practice [8].

HPLC works by passing a sample through a column, where its components separate based on how they interact with a solid stationary phase and a liquid mobile phase, allowing them to exit one by one [9].

METERIALS AND METHODS

Pig bone marrow was obtained from the V.C.N. piggery farming in Pudukkottai. Selected antidepressant drugs were purchased from an authorized medical shop in Pudukkottai. The bone marrow and drugs were submitted to the Forensic Science and Criminology Department at Annai Fathima College of Arts and Science in Madurai. The assistant professor identified and verified the samples. The collected tablets were stored at room temperature in a dry place until further analysis. The bone marrow sample was stored in a freezer at a temperature of –20 °C until further analysis.

Standard of drug free bone marrow:

The bone marrow sample was removed from the −20 °C freezer. Then, 1.0 g of blank bone marrow was carefully weighed into centrifuge tubes, and 2 mL of methanol was added. The mixture was thoroughly vortexed for 5 minutes and centrifuged at 6,000 rpm for 10 minutes. Finally, the clear supernatant was gently collected and transferred into an HPLC vial for further analysis.

Standard of drug sample:

Antidepressant tablets containing amitriptyline hydrochloride and imipramine hydrochloride (25 mg each) were crushed into a fine powder, and a portion equivalent to 10 mg of the drug was dissolved in methanol. This solution was prepared as the stock by transferring it into a 10 mL volumetric flask and ensuring complete dissolution. A working solution was then made by diluting 1 mL of the stock solution up to 10 mL with methanol. For the analysis, 1 mL of the working solution (containing 0.5 mL of each drug) was placed in an HPLC vial for further analysis.

Sample solution preparation:

The acetate buffer was prepared by dissolving 78 mg of sodium acetate in 15 mL of distilled water with continuous stirring until completely dissolved. Then, 32 µL of glacial acetic acid was added slowly dropwise, and the pH was checked after proper mixing. For sample preparation, 1 g of bone marrow was taken and mixed with 3 mL of the prepared buffer, followed by vortexing for 3 minutes. After that, 50 µL of each drug (amitriptyline and imipramine) working solution was added, and the mixture was vortexed again to ensure proper mixing.

The samples were then kept for equilibration to allow the drugs to interact and bind with the bone marrow tissue. One set of samples was stored at 4 °C for 1 hour, while others were stored at −20 °C for 72 hours and 5 days to maintain stability and prevent degradation. For extraction, an organic solvent mixture of chloroform and isopropyl alcohol (15 mL and 5 mL) was prepared and mixed well. After equilibration, 1 mL of potassium hydroxide was added to the sample and vortexed, followed by the addition of 5 mL of the organic solvent, vortexing, and centrifugation to separate the layers.

The organic layer was carefully collected and evaporated in a water bath at 40-45 °C until a dry residue was obtained. Finally, the residue was reconstituted with 200 µL of methanol and vortexed to obtain the final sample, which was placed in an HPLC vial for further analysis.

The instrument used in this study was High-Performance Liquid Chromatography with a Diode Array Detector (HPLC-DAD), chosen for its high sensitivity, accuracy, and ability to separate and measure drugs in bone marrow samples. It also helps in

identifying compounds by analysing their UV spectra at multiple wavelengths. The method works by injecting the sample into a flowing mobile phase, which carries it through a column containing a stationary phase, where separation takes place.

The system includes a solvent reservoir to hold the mobile phase, a high-pressure pump to maintain a steady flow, and an injector for accurate sample introduction. The column, kept at a controlled temperature, ensures consistent separation of compounds. As the separated components leave the column, they pass through the diode array detector, where light from a deuterium or tungsten lamp passes through the sample. The detector measures absorbance across different wavelengths simultaneously. Finally, the data is processed using software to produce chromatograms and spectra, aiding in the identification, purity assessment, and quantification of the drugs.

RESULT

This analysis evaluates the concentration of Amitriptyline and Imipramine in a bone marrow matrix over a 120-hour(5day) period.

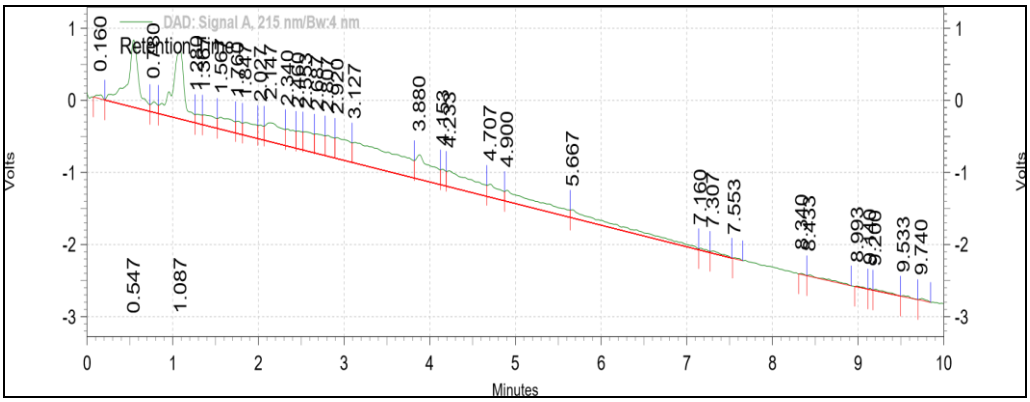

Figure-1: HPLC analysis- drug-free bone marrow

The chromatogram displays the HPLC analysis of drug-free bone marrow using a diode array detector at 215 nm. A slight downward trend in the baseline is observed, which may be due to the increasing amount of methanol in the mobile phase during the run. Several peaks appear in the chromatogram, primarily from natural components like fats, proteins, and amino acids present in the bone marrow. This confirms that no drug is present in the sample.

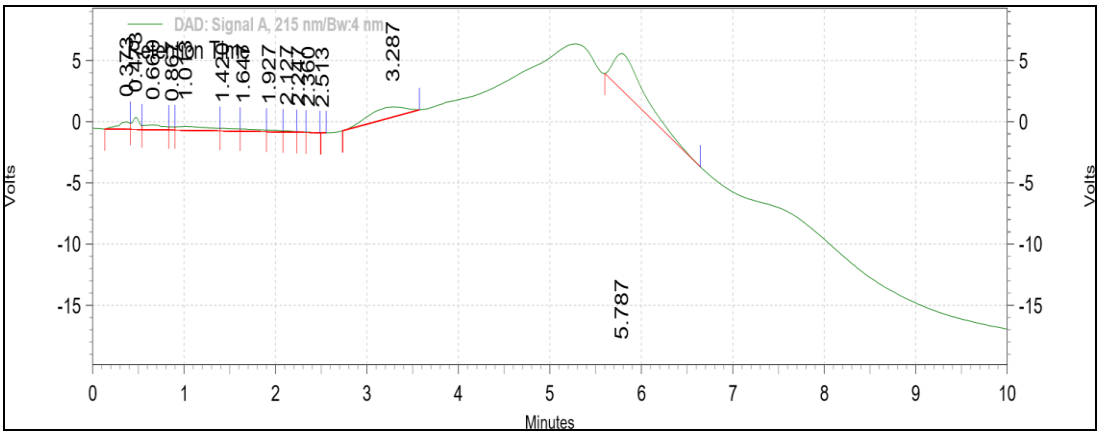

Figure-2: HPLC analysis- standard drug

The chromatogram shows the HPLC analysis of the standard mixture of amitriptyline and imipramine using a diode array detector at 215 nm. A single peak is observed at 5.787 minutes, representing the drug standard. Separate peaks are not clearly seen because both drugs have very similar structures and tend to elute together. This peak was used as a reference for comparing the test samples.

Journal of Current Pharma Research (JCPR)
Volume 22, Issue 4, April 2026 **jcpr.humanjournals.com** ISSN: 2230-7842, 2230-7834

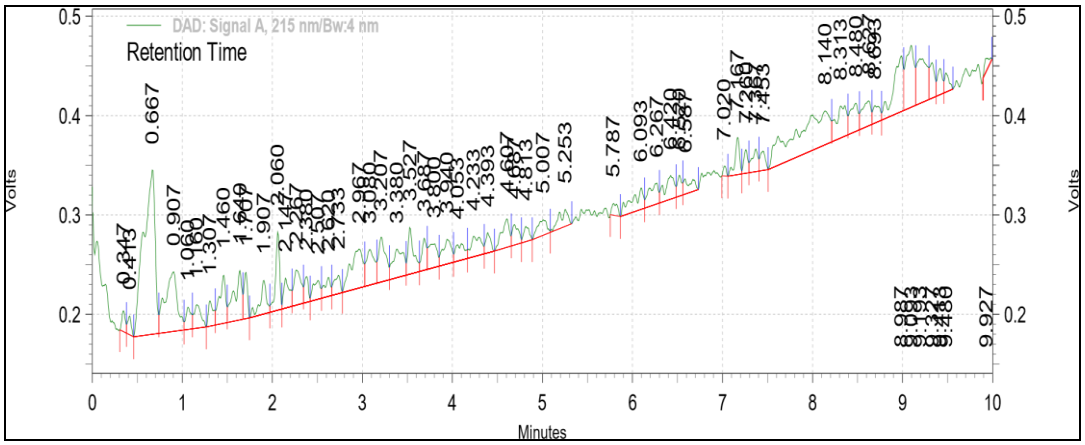

Figure-3: HPLC analysis- 0th day

The chromatogram shows the HPLC analysis of spiked bone marrow on the 0th day after a 1-hour equilibration using a diode array detector at 215 nm. The baseline shows a gradual upward increase compared to the blank and standard runs. A small peak is clearly seen at 5.787 minutes, matching the drug standard. This indicates that the drugs were successfully extracted from the bone marrow sample.

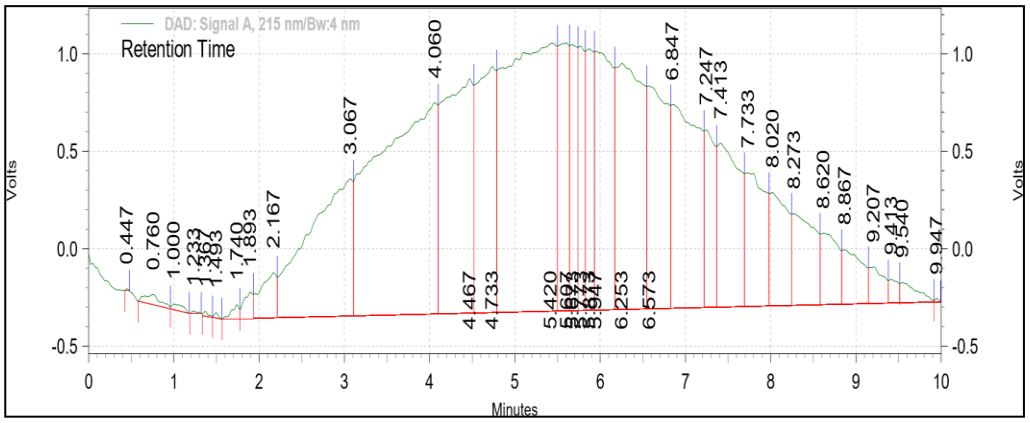

Figure-4: HPLC analysis- 3rd day

The chromatogram shows the HPLC analysis of spiked bone marrow on the 3rd day after 72 hours of storage at −20 °C using a diode array detector at 215 nm. The original drug peak at 5.787 minutes is no longer clearly visible, suggesting a reduction in the parent drug. Instead, a small peak appears around 5.840 minutes. This may be due to the formation of a degradation product or metabolite over time.

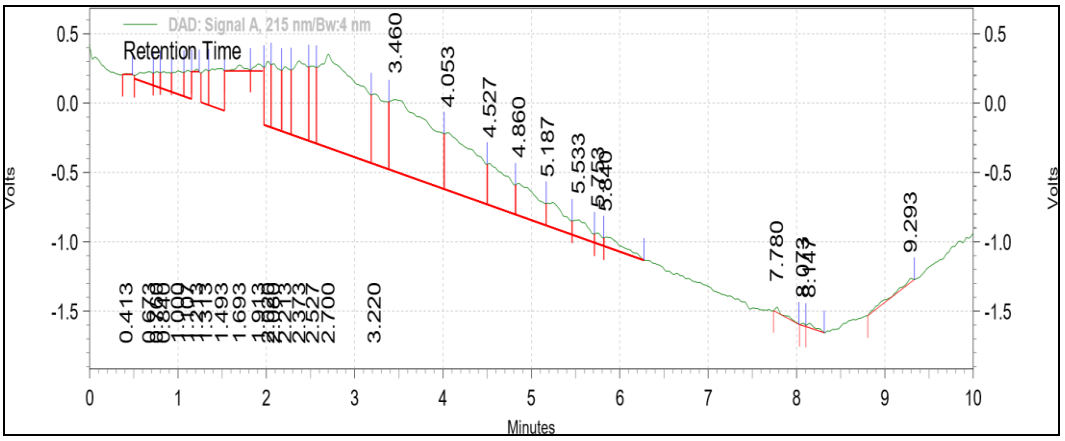

Figure-5: HPLC analysis- 5th day

The chromatogram shows the HPLC analysis of spiked bone marrow on the 5th day after 120 hours using a diode-array detector at 215 nm. Unlike earlier results, no clear drug peak is seen at 5.787 minutes or near 5.840 minutes. This indicates that the drugs are no longer detectable in the sample. It suggests that amitriptyline and imipramine have degraded or been lost over time.

The HPLC data, including peak integration, were analysed using OriginPro to obtain clear and reliable results. The software helps in correcting the baseline, fitting peaks properly, and performing basic statistical analyses. It also makes it easier to compare different samples and observe changes in peak and baseline patterns. In addition, it allows the creation of simple and clear graphs for a better understanding of the data.

Quantity: It represents how much of a substance is detected by the detector at a specific retention time.
Time: It refers to the time taken for a compound to pass through the column and reach the detector.

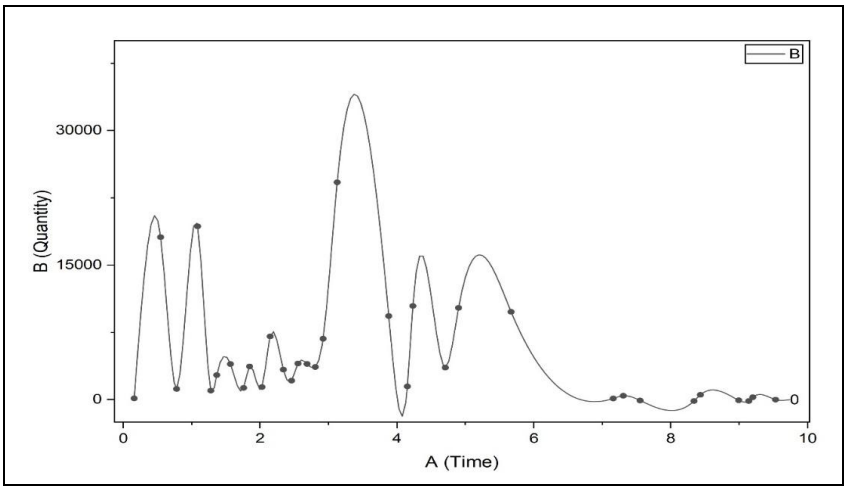

Figure-6: Processed chromatogram- drug-free bone marrow

X-axis represents retention time.

Y-axis represents the quantity.

- This symbol represents the detector response the sample substance.

The processed chromatogram of drug-free bone marrow was obtained using OriginPro by plotting area against retention time and adjusting the baseline to make it flat. Many peaks are seen between 0.5 and 6.0 minutes, indicating that bone marrow is a complex and noisy sample. No drug peaks are present in this chromatogram. A small negative dip around 4 minutes may be due to a mismatch between the sample solvent and the mobile phase.

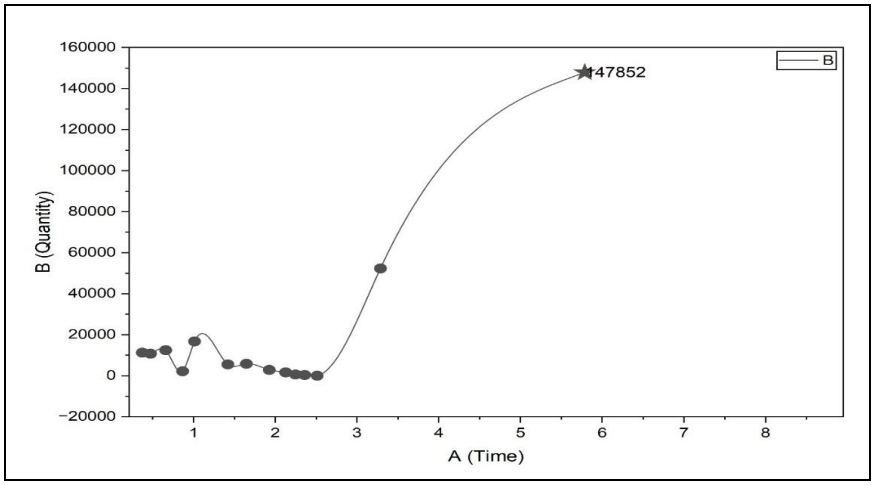

Figure-7: Processed chromatogram- Standard drug

● - This symbol represents the detector response the sample substance.

★ -This symbol represents the detector response of the drug (amitriptyline and imipramine) sample.

The processed chromatogram of the drug standards (amitriptyline and imipramine) was obtained using OriginPro by plotting the area against retention time and adjusting the baseline. An absorbance is seen at 5.787 minutes, representing the drug standard. The peak area of the drug is 147,852.

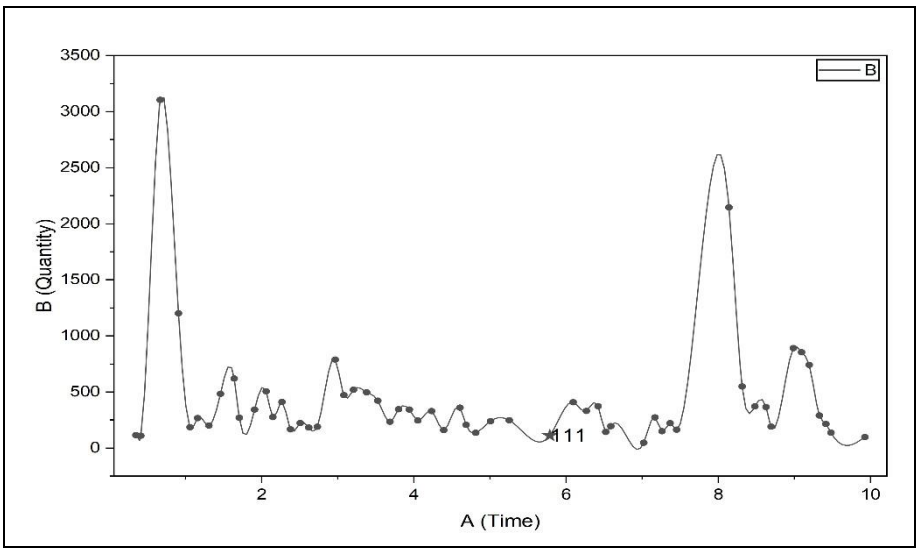

Figure-8: Processed chromatogram- 0th day sample

● - This symbol represents the detector response the sample substance.

★ - This symbol represents the detector response of the drug (Amitriptyline and Imipramine) sample.

The processed chromatogram of the 0th-day sample (1-hour equilibration) was obtained using OriginPro with a flat baseline. An absorbance is seen at 5.787 minutes, similar to the standard drug. The peak area of drug absorbance 111.This shows that the drug was successfully extracted from the bone marrow.

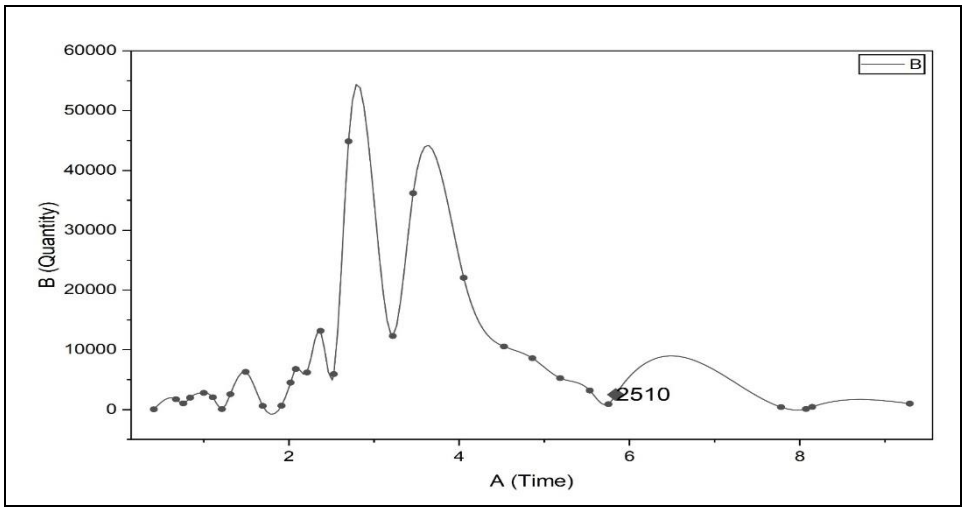

Figure-9: Processed chromatogram- 3rd day sample

● - This symbol represents the detector response the sample substance.

◆ - This symbol represents the detector response of the drug (This could be a metabolite or a degradation product).

The processed chromatogram of the 3rd day sample (72-hour equilibration) was obtained using OriginPro with a flat baseline. The absorbance at 5.787 minutes seen earlier is no longer present. Instead, an absorbance appears at 5.840 minutes. This may indicate the formation of a degradation product with slightly different properties than those of the original drug.

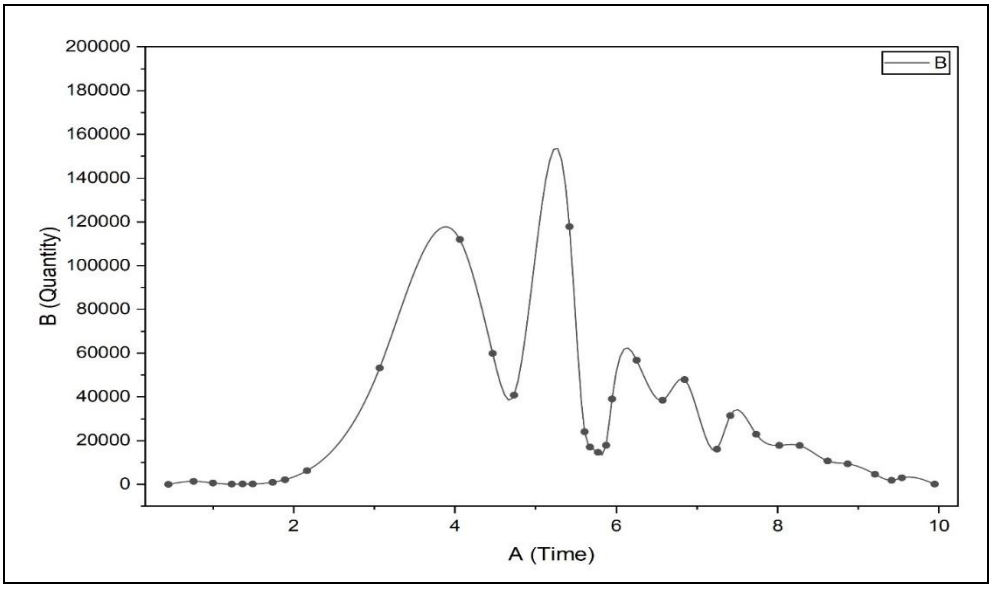

Figure-10: Processed chromatogram- 5th day sample

- This symbol represents the detector response of the sample substance.

The processed chromatogram of the 5th-day sample (120-hour equilibration) was obtained using OriginPro with a flat baseline. No absorbance is seen at 5.787 minutes, as observed earlier. This shows that the drugs are no longer detectable in the sample.

CONCENTARTION OF THE SAMPLE:

Sample of the concentration=sample of the area/ standard of the area * Standard of the concentration.

0th day sample concentration:

sample of the area= 111

standard of the area=147852

Standard of the concentration= 100 µg/mL

Sample of the concentration= 111/147852 * 100 ~0.075 µg/mL.

Sample of the concentration= 0.075 µg/mL.

Recovery of the sample (%) = found dug amount / spiked drug amount * 100

= 0.075 / 50 * 100

Recovery of the sample (%) = 0.15%

very small amount of the drug was recovered.

3rd day sample concentration:

The concentration could not be determined because, the parent drug was not recovered in 3rd day sample.

5th day sample concentration:

The concentration could not be determined because, the parent drug was not recovered in 5th day sample.

DISCUSSION

Our research was related to the drug concentration in postmortem bone marrow. The standard results for the two drugs are not absorbed separately: Imipramine and Amitriptyline. Because these two drugs are structurally very similar (tricyclic antidepressants), they often elute very close together. In this HPLC result, they appear to be co-eluting. On the 0th day (1 hour), the substance absorbed at 5.787 minutes. The same as the standard drug analysis, the drug substance absorbed at 5.787 minutes. The quantity of the drugs was comparatively much lower than the standard drug. This represents the lower concentration of the recovered drugs, proving that the drugs were successfully extracted from the bone marrow. However, the concentration of the recovered drugs was lower. On the 3rd day (72 hours), the equilibration substance absorbed at 5.840 minutes. In the standard drug analysis, the drug substance absorbed at 5.787 minutes. This indicates that there could be a degradation product with a very similar chemical structure to the original drug but slightly different polarity. Therefore, we can't calculate the concentration of this sample. The 5th day (120 hours) result showed no absorbed substance at that specific time. It proves that the parent drugs (Amitriptyline and Imipramine) are undetectable. Consequently, we can't calculate the concentration of this sample. The absorbance of the substance in the bone marrow sample is due to endogenous compounds. These are naturally occurring proteins, lipids, or small molecules within the marrow.

The previous studies, such as Chang, Y. Y., et al. (2021), conducted an experiment on the simultaneous determination of ten anti-tumour drugs in different biological samples. They used biological samples (human plasma and urine), spiked with multiple anti-tumour drugs, and analysed them using HPLC-DAD. They compared three advanced chemometric methods to accurately resolve overlapping peaks and correct time shifts in chromatographic data. They concluded that chemometric techniques enable accurate multi-component drug determination in complex and noisy biological systems. While this study found that the selected two antidepressant drugs (amitriptyline and imipramine), biological sample (bone marrow), and spiked with two drugs were analysed using HPLC-DAD, the HPLC-DAD results showed that the absorbance of imipramine and amitriptyline overlapped. This issue may be due to the absence of chemometric data processing techniques. As a result, quantification of individual drugs becomes challenging.

CONCLUSION

This study successfully analysed HPLC-DAD to detect tricyclic antidepressants (TCAs) within the complex biological matrix of bone marrow. Drug-free bone marrow analysis revealed numerous peaks, confirming the presence of endogenous substances like fats, proteins, and amino acids. In the drug standard analysis, due to these two drugs (amitriptyline and imipramine) being structurally very similar, the substances co-eluted at a retention time of 5.787 minutes, representing the total tricyclic antidepressants (TCAs) presence rather than individual peaks. The analysis of the 1-hour (0th day) sample confirmed that amitriptyline and imipramine can be successfully extracted from bone marrow tissue. The study identified a significant instability of the parent drugs over time, with the parent drug (original chemical signature) disappearing within 72 hours. By the 3rd day, the disappearance of the 5.787-minute peak and the emergence of a new peak at 5.840 minutes suggest that the parent drugs have decreased or degraded. The slight shift in retention time (from 5.787 to 5.840) indicates that the degradation products possess a different polarity than the parent drugs while maintaining a similar core structure. By the 5th day (120 hours), the parent drugs were completely undetectable, proving that these drugs are not stable in bone marrow. Concentration could only be accurately determined for the 0th day sample (0.075 µg/mL); subsequent samples were unquantifiable due to chemical transformation. These findings suggest that in the early stages of decomposition in forensic cases, the parent drugs (amitriptyline and imipramine) may not be detectable in bone marrow samples. This finding shows that it is important to look for metabolites and to consider the unique challenges of the bone marrow matrix when performing forensic toxicological analysis.

ACKNOWLEDGEMENT

My sincere gratitude goes out to ANNAI FATHIMA COLLEGE OF ARTS AND SCIENCE, THIRUMANGALAM, MADURAI for providing the necessary facilities, guidance, and support to carry out this research work. I would like to express my sincere thanks to the LADY DOAK COLLEGE, MADURAI for providing the HPLC-DAD instrument essential to the completion of this study. I would like to express my sincere gratitude to the V.C.N Piggery farming, Pudukkottai for providing the bone marrow samples essential to the completion of this study. Finally, I would like to express my heartfelt gratitude to my parents and friends for supporting me in the completion of this research.

REFERENCES

1. Travlos, G. S. (2006). Normal structure, function, and histology of the bone marrow. Toxicologic pathology, 34(5), 548-565.

2. Kumar, R., Godavarthy, P. S., & Krause, D. S. (2018). The bone marrow microenvironment in health and disease at a glance. *Journal of Cell Science*, *131*(4), jcs201707
3. Gerdts, V., Wilson, H. L., Meurens, F., van Drunen Littel-van den Hurk, S., Wilson, D., Walker, S., ... & Potter, A. A. (2015). Large animal models for vaccine development and testing. *ILAR journal*, *56*(1), 53-62.
4. World Health Organization. (2018). *New WHO guidelines to improve the physical health of people with severe mental disorders.*
5. Berm, E. J., Paardekooper, J., Brummel-Mulder, E., Hak, E., Wilffert, B., & Maring, J. G. (2015). A simple dried blood spot method for therapeutic drug monitoring of the tricyclic anti-depressant's amitriptyline, nortriptyline, imipramine, clomipramine, and their active metabolites using LC-MS/MS. *Talanta*, *134*, 165-172.
6. Dopheide, J. A. (2006). Recognizing and treating depression in children and adolescents. *American journal of health-system pharmacy*, *63*(3), 233-243.
7. Stokes, P. R., Jokinen, T., Amawi, S., Qureshi, M., Husain, M. I., Yatham, L. N., ... & Young, A. H. (2020). Pharmacological treatment of mood disorders and comorbid addictions: a systematic review and meta-analysis: traitement pharmacologique des troubles de l'humeur et des dépendances comorbides: une revue systématique et une méta-analyse. *The Canadian Journal of Psychiatry*, *65*(11), 749-769.
8. Hiemke, C., Baumann, P., Bergemann, N., Conca, A., Dietmaier, O., Egberts, K., ... & Zernig, G. (2011). AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011. *Pharmacopsychiatry*, *21*(06), 195-235.
9. Aryal, S., PhD. (2025, June 14). *HPLC: Principle, parts, Types, Uses, Diagram*. Microbe Notes. https://microbenotes.com/high-performance-liquid-chromatography-hplc/
10. Giordano, G. (2025). FORENSIC TOXICOLOGY IN UNCONVENTIONAL BIOLOGICAL MATRICES: THEEMERGING ARCHAEOTOXICOLOGICAL FIELD APPLIED TO BONE REMAINS ANDBRAIN TISSUE IN ANCIENT POPULATIONS.
11. Dalsasso, L. C. F., & Marchioni, C. (2024). Post-mortem toxicological analysis of cocaine: main biological samples and analytical methods. *Forensic Science, Medicine and Pathology*, *20*(3), 1091-1101.
12. Kumar, S., Darshan, S., & Baggi, T. R. (2023). Recent advances in analytical techniques for antidepressants determination in complex biological matrices: a review. *International journal of* toxicology, *42*(4), 352-364.
13. Soares, S., Barroso, M., & Gallardo, E. (2021). A review of current bioanalytical approaches in sample pretreatment techniques for the determination of antidepressants in biological specimens. *Reviews in Analytical Chemistry*, *40*(1), 12-32.
14. Chang, Y. Y., Wu, H. L., Fang, H., Wang, T., Ouyang, Y. Z., Sun, X. D., ... & Yu, R. Q. (2021). Comparison of three chemometric methods for processing HPLC-DAD data with time shifts: Simultaneous determination of ten molecular targeted anti-tumor drugs in different biological samples. *Talanta*, *224*, 121798.

How to cite this article:
N. Priyadharshini et al. Jcpr.Human, 2026; Vol. 22 (4): 9-17

Conflict of Interest Statement:
The authors have no conflicts of interest to declare.