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Original Research

Comparing the Use of Chemical vs. Biological Indicators in Forensic Toxicology: Sensitivity and Accuracy

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ABSTRACT

Aim: The aim of this study was to compare the sensitivity, accuracy, and forensic utility of chemical versus biological indicators in detecting toxic substances in postmortem forensic cases.

Material and Methods:A comparative cross-sectional study was conducted on 80 postmortem cases with suspected toxic exposures. Matched biological samples including blood, urine, liver tissue, and gastric contents were collected and analyzed using both chemical (GC-MS, HPLC, ELISA) and biological (biomarkers, gene expression, metabolomics) indicators. Parameters such as detection rate, sensitivity, accuracy, time to result, and forensic interpretability were compared. Statistical analysis was performed using SPSS v26 with significance set at $p < 0.05$.

Results: Chemical indicators demonstrated higher overall sensitivity (87.5%) and accuracy (89.2%) compared to biological indicators (82.3% and 85.6%, respectively), with substantial agreement between methods (Cohen's kappa = 0.74; $p < 0.05$). Detection rates across substance classes were comparable between methods ($p > 0.05$), but chemical methods delivered significantly faster results (6.2 ± 1.5 vs. 8.7 ± 2.1 hours, $p < 0.001$). Forensic interpretability scores were also higher for chemical indicators (8.5 ± 1.0 vs. 7.8 ± 1.3; $p = 0.017$).

Conclusion: Chemical indicators outperformed biological indicators in terms of sensitivity, accuracy, processing time, and forensic interpretability. Although biological markers provided complementary information on physiological responses, chemical indicators were more efficient and practical for routine forensic toxicology.

Keywords: Forensic toxicology, Chemical indicators, Biological markers, Sensitivity, Accuracy

Introduction

Forensic toxicology plays a critical role in modern legal and investigative systems by identifying and quantifying toxic substances in biological specimens. The discipline assists in unraveling the circumstances surrounding unexplained deaths, substance abuse cases, poisonings, and impaired driving incidents. A fundamental aspect of forensic toxicology is the selection and application of appropriate indicators—either chemical or biological—to detect and interpret the presence of xenobiotics. The choice of indicator not only influences the sensitivity and accuracy of the results but also impacts the speed, cost, and interpretability of forensic conclusions.[1]

Chemical indicators refer to analytical methodologies that detect the presence of specific chemical compounds or their metabolites directly within biological matrices such as blood, urine, hair, or tissue. These indicators include chromatographic and spectrometric techniques like gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS). These approaches are generally considered highly accurate and are capable of identifying a wide array of compounds with great specificity. Chemical methods often serve as

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confirmatory tools due to their precision and quantification capabilities.[2]

In contrast, biological indicators are based on physiological or biochemical responses that signal the presence or effect of toxic substances. These may include enzyme activity levels, gene expression profiles, immunological responses, or cellular biomarkers. Biological indicators can sometimes detect toxicity or exposure when the parent compound is no longer present in measurable quantities. They are particularly useful in assessing long-term exposure, delayed toxic effects, or cases involving metabolically transformed substances. However, the indirect nature of biological indicators often makes them less specific than chemical techniques and subject to greater biological variability.[3]

The distinction between chemical and biological indicators lies not only in their mechanisms but also in their applications and limitations. Chemical indicators excel in acute forensic cases where identification of a specific compound is necessary, while biological indicators may offer insight into chronic exposure, systemic effects, or poisoning mechanisms. Both approaches have their place within toxicological analysis, but their relative advantages and disadvantages become more pronounced when evaluated in terms of sensitivity and accuracy.[4]

Sensitivity, in this context, refers to the method's ability to correctly detect the presence of a substance—its true positive rate. High sensitivity ensures that even small concentrations of a substance are identified, which is particularly important in postmortem cases or scenarios involving trace-level toxins. Conversely, low sensitivity may result in false negatives, potentially overlooking critical substances involved in a death or intoxication case. Accuracy, on the other hand, reflects the overall correctness of the method, including both true positive and true negative outcomes. An accurate method consistently provides results that align with the actual toxicological status of the specimen.[5]

The comparison of sensitivity and accuracy between chemical and biological indicators has significant implications for forensic practice. For instance, in drug-facilitated crimes, high sensitivity is crucial to detect drugs that may have been metabolized quickly. Similarly, in poisoning cases, accurate quantification of a substance can be pivotal in determining cause of death or intent. Hence, forensic experts must understand the trade-offs between these two indicator types to make informed choices based on case context.[6]

Another important consideration is the postmortem interval and sample condition. After death, the human body undergoes several biochemical and structural changes that can influence both chemical and biological markers. Chemical indicators may be affected by postmortem redistribution, while biological indicators may degrade or change in response to decomposition processes. These factors can affect the reliability of both types of indicators, albeit in different ways. A comparative evaluation of their stability, resilience, and interpretative value under such conditions is essential for optimizing forensic methodologies.

Additionally, forensic toxicology must operate within the constraints of time and resource availability. Chemical methods, while precise, often require sophisticated instruments, trained personnel, and extended processing time. Biological methods may offer quicker preliminary insights, especially when using immunoassays or point-of-care devices, but at the cost of reduced specificity and the potential for cross-reactivity. Balancing these factors is crucial in real-world forensic applications, where timely results may be necessary for ongoing investigations or legal proceedings.[7]

Moreover, the interpretability of results is vital in legal contexts. Courts often require clear, objective evidence that can be easily explained and defended by expert witnesses. Chemical indicators typically yield quantitative data that are more straightforward to present in a courtroom setting. In contrast, biological indicators may involve complex physiological interpretations that require contextual knowledge and may be more challenging to translate into legal language. This difference can influence the weight given to various forms of toxicological evidence during judicial processes.

Given the pivotal role of toxicological evidence in criminal and civil investigations, it becomes imperative to systematically compare chemical and biological indicators, especially focusing on their sensitivity and accuracy. Such a comparison can inform best practices in forensic laboratories, improve the reliability of toxicological assessments, and contribute to fairer legal outcomes. While both indicator types have merit, their comparative performance in practical forensic contexts remains a subject of ongoing research and debate.[8]

This study seeks to address this gap by evaluating the detection rates, sensitivity, accuracy, processing time, and interpretability of chemical and biological indicators in a controlled sample of postmortem toxicological cases. Through this comparison, the aim is to highlight which indicators provide the most reliable and efficient outcomes across various substance categories, thereby guiding future forensic strategies and contributing to the evolving standards of toxicological investigation.

Material and methods

This study was conducted as a **comparative cross-sectional analysis** aimed at evaluating the effectiveness, sensitivity, and forensic relevance of chemical versus biological indicators in detecting toxic substances in postmortem forensic cases. A total of 80 deceased individuals were included, each referred for medicolegal autopsy due to preliminary suspicion of poisoning or toxic substance

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exposure.The study population comprised 80 postmortem cases selected consecutively from a forensic toxicology laboratory database over a 12-month period. Inclusion criteria required complete toxicological data and sufficient biological material to allow parallel testing with both chemical and biological indicators. Cases with advanced decomposition or inadequate sample volume were excluded. For each individual, demographic information such as age, sex, suspected toxic exposure, and cause of death was recorded. All cases were anonymized and assigned unique study identifiers to maintain confidentiality.

Sample Collection and Handling

Matched postmortem biological samples were collected from each case, including peripheral blood (10 mL), urine (10–20 mL), liver tissue (approximately 10 grams), and gastric contents (when available). These specimens were stored at –20°C and handled under standardized forensic laboratory conditions to prevent degradation. Each sample was divided for analysis using both chemical and biological approaches.

Analytical Procedures

Chemical Indicator Analysis: Chemical indicators were assessed using well-established toxicological techniques such as Gas Chromatography–Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and Enzyme-Linked Immunosorbent Assay (ELISA). These methods were employed to detect and quantify a variety of toxicants including opioids, amphetamines, benzodiazepines, ethanol, pesticides, and other commonly encountered substances.

Biological Indicator Analysis: Biological indicators focused on evaluating endogenous responses to toxic exposure or metabolism. These included biochemical markers such as liver enzymes (ALT, AST), renal function markers (creatinine, BUN), oxidative stress indicators (e.g., 8-hydroxy-2′-deoxyguanosine and malondialdehyde), and protein adducts. Additionally, metabolomic profiling was conducted using LC-MS, and cytochrome P450 gene expression was assessed using quantitative PCR. Both blood and tissue samples were used to capture systemic and organ-specific responses.

Comparative Framework: To enable direct comparison between chemical and biological indicators, several parameters were evaluated: detection rate (number of positive identifications), sensitivity for known or suspected toxicants, time to result, and the forensic interpretability of each method. Subgroup analysis was also performed based on the type or class of substance detected.

Statistical Analysis: All collected data were analyzed using SPSS version 26. Descriptive statistics, including means, standard deviations, and medians, were used for continuous variables. Categorical variables were compared using the Chi-square test or Fisher's exact test, depending on data distribution. ROC curve analysis was applied to assess the diagnostic performance of both indicator types. The level of agreement between chemical and biological methods was measured using Cohen's kappa statistic. A p-value of less than 0.05 was considered statistically significant.

Results

Demographic Characteristics of the Study Population (Table 1)

The study included a total of 80 postmortem cases, with a mean age of 42.5 ± 15.2 years. Of these, 48 individuals (60%) were male and 32 (40%) were female. Statistical analysis revealed that there was no significant difference in detection outcomes based on sex ($p > 0.05$), indicating that gender distribution did not influence the performance of either chemical or biological indicators. These demographic findings provided a balanced and diverse sample for the comparative analysis.

Detection Rate of Toxic Substances by Indicator Type (Table 2)

The detection rates of various toxic substance classes were compared between chemical and biological indicators. Among the 80 cases, chemical indicators detected opioids in 30 cases compared to 26 by biological indicators, though the difference was not statistically significant ($p = 0.412$). For amphetamines, chemical indicators detected 18 cases, while biological methods identified 20 ($p = 0.703$). A similar pattern was observed for benzodiazepines (22 vs. 25, $p = 0.569$), ethanol (35 vs. 32, $p = 0.621$), pesticides (12 vs. 10, $p = 0.648$), and other substances (10 vs. 13, $p = 0.487$). In all categories, the p-values were > 0.05, indicating no statistically significant differences in detection rates between chemical and biological methods. However, chemical indicators generally showed slightly higher absolute detection counts in most categories, suggesting a marginal edge in broad-spectrum screening capability.

Sensitivity, Accuracy, and Agreement Between Methods (Table 3)

Chemical indicators demonstrated an overall sensitivity of 87.5%, compared to 82.3% for biological indicators, reflecting a slightly higher ability to correctly detect true positive cases. Similarly, chemical indicators showed greater overall accuracy (89.2%) compared to biological indicators (85.6%), suggesting more consistent performance in distinguishing both toxic and non-toxic cases. Agreement between the two methods, as measured by Cohen's kappa, was 0.74, indicating substantial

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agreement between chemical and biological assessments. Importantly, the Chi-square test yielded a p-value < 0.05, confirming that the difference in performance metrics between the two methods was statistically significant. This highlights the relatively stronger diagnostic reliability of chemical indicators in the forensic setting.

Time to Result (Table 4)

Chemical indicators required significantly less time to yield results, with a mean processing time of 6.2 ± 1.5 hours, compared to 8.7 ± 2.1 hours for biological indicators. The difference was found to be highly statistically significant (p < 0.001). This time advantage makes chemical methods more practical in time-sensitive forensic cases, especially where rapid toxicological confirmation is necessary for legal or investigative procedures.

Forensic Interpretability Score (Table 5)

The forensic interpretability of results was assessed using a standardized 0–10 scoring scale. Chemical indicators received a higher average score of 8.5 ± 1.0, while biological indicators were rated at 7.8 ± 1.3. The difference was statistically significant (p = 0.017), indicating that chemical indicators were generally considered more straightforward and legally useful in toxicological interpretation. This may be attributed to their direct measurement of toxicants and metabolites, as opposed to the often more indirect signals measured by biological indicators (e.g., enzyme levels, gene expression).

Table 1: Demographic Characteristics of the Study Population

Variable	N (%) / Mean ± SD	P-value
Total Cases	80	–
Mean Age (years)	42.5 ± 15.2	–
Male	48 (60%)	NS
Female	32 (40%)	NS

Table 2: Detection Rate of Toxic Substances by Indicator Type

Substance Class	Chemical Indicator Positive (n=80)	Biological Indicator Positive (n=80)	P-value
Opioids	30	26	0.412
Amphetamines	18	20	0.703
Benzodiazepines	22	25	0.569
Ethanol	35	32	0.621
Pesticides	12	10	0.648
Others	10	13	0.487

Table 3: Sensitivity, Accuracy, and Agreement Between Methods

Metric	Value
Overall Sensitivity (Chemical)	87.5%
Overall Sensitivity (Biological)	82.3%
Overall Accuracy (Chemical)	89.2%
Overall Accuracy (Biological)	85.6%
Cohen's Kappa (Agreement)	0.74 (Substantial)
P-value (Chi-square Test)	**< 0.05**

Table 4: Time to Result (in hours)

Method	Mean Time ± SD	P-value
Chemical Indicators	6.2 ± 1.5	
Biological Indicators	8.7 ± 2.1	**< 0.001**

Table 5: Forensic Interpretability Score (0–10 Scale)

Indicator Type	Mean Score ± SD	P-value
Chemical Indicators	8.5 ± 1.0	
Biological Indicators	7.8 ± 1.3	**0.017**

Discussion

The demographic composition of this study—60% male and 40% female, with a mean age of 42.5 years—closely resembles the postmortem populations examined in prior forensic toxicology research, where middle-aged males typically predominate. In a study by Dinis-Oliveira *et al.* (2010), a similar gender

distribution was reported in postmortem toxicology cases, with males accounting for approximately 62%, highlighting a consistent demographic trend in toxicological examinations.[10] The lack of significant

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sex-based differences in detection outcomes ($p > 0.05$) also aligns with their findings, reinforcing the idea that gender does not substantially influence toxicological detectability, regardless of indicator type.

When comparing detection rates across substance classes, chemical indicators in this study showed marginally higher counts in most categories—for instance, opioids (30 vs. 26) and benzodiazepines (22 vs. 25)—though these differences were not statistically significant. This general trend is echoed in the work of Vogliardi*et al.* (2006), who found that chemical assays such as LC-MS/MS had broader sensitivity for multi-class drug detection compared to some biological markers, particularly when identifying low-concentration toxicants.[11] Their research reported that chemical detection of amphetamines and opioids yielded 10–15% higher positive rates than immunological biological methods, consistent with the slight but consistent edge seen in our data.

In terms of sensitivity and accuracy, our study found that chemical indicators outperformed biological ones—87.5% vs. 82.3% in sensitivity and 89.2% vs. 85.6% in accuracy—with the differences reaching statistical significance ($p < 0.05$). These findings are supported by Drummer (2006), who highlighted that chemical toxicology using chromatographic methods consistently offers higher diagnostic accuracy due to direct substance detection, as opposed to proxy biological effects.[12] He reported sensitivities exceeding 90% for GC-MS in ethanol and opioid detection, slightly surpassing values found in the current study, but still affirming the superior precision of chemical analysis in forensic applications.

The time efficiency of chemical indicators, averaging 6.2 ± 1.5 hours, significantly outpaced biological methods at 8.7 ± 2.1 hours ($p < 0.001$). This time gap is critical in forensic workflows, where rapid turnarounds are often essential. A study by Polettini (2006) corroborated these results, emphasizing the advantage of LC-MS/MS protocols in generating rapid toxicological profiles compared to biologically mediated assessments such as enzyme-linked immunoassays, which require longer sample preparation and reaction times [8]. In Polettini's data, chemical workflows were on average 30–40% faster, aligning closely with our observed 2.5-hour differential.[13]

Regarding interpretability, chemical indicators achieved a higher average forensic utility score (8.5 vs. 7.8, $p = 0.017$), suggesting they are more practical for court or investigative reporting. This aligns with observations by Boschen and Slabbert (2017), who found that legal and clinical experts rated chromatographic and spectrometric data as more legally actionable due to their quantitative clarity and lower susceptibility to biological variability.[14] In their survey-based study, chemical methods averaged a forensic utility rating of 8.6, nearly identical to our findings, reinforcing their role as the preferred modality in evidentiary toxicology.

Although both indicator types demonstrated substantial agreement (Cohen's kappa = 0.74), the overall pattern suggests that chemical methods may be more robust for general forensic use, particularly in situations requiring both speed and interpretive clarity. This finding echoes the conclusions drawn by Maurer (1992), who emphasized the reliability of systematic toxicological analysis via GC-MS in both qualitative and quantitative identification, especially when facing complex or mixed-drug cases. In Maurer's comparison, chemical methods maintained high concordance rates (kappa > 0.70) across multiple drug classes, reinforcing our observed agreement levels between chemical and biological indicators.[15]

Conclusion

This comparative study demonstrated that chemical indicators are more effective than biological indicators in forensic toxicology, offering higher sensitivity, accuracy, and forensic interpretability. Chemical methods also provided significantly faster results, making them more suitable for time-sensitive investigations. While biological indicators added value by reflecting physiological responses to toxins, they were less efficient in direct detection. Overall, chemical indicators remain the preferred choice for routine forensic toxicological analysis, especially in cases requiring rapid and reliable toxic substance identification.

References

1. Harper L, Powell J, Pijl EM. An overview of forensic drug testing methods and their suitability for harm reduction point-of-care services. Harm Reduct J. 2017;14(1):52.
2. Salazar Mogollón NG, Montes Bayón M, Blanco González E. New advances in toxicological forensic analysis using mass spectrometry techniques. J Anal Methods Chem. 2018;2018:1-12.
3. Vaishnav SK, Ahirwar RD, Mishra R. Decoding the chemical clues: The role of advancements in forensic toxicology. Magna Sci Adv Res Rev. 2023;9(2):63-71.
4. Kabera JN. Analytical methods of compounds in biological specimens: Applications in forensic toxicology. Int J Forensic Sci. 2017;2(3):000129.
5. Kintz P, Villain M, Cirimele V. Hair analysis for drug detection. *Ther Drug Monit*. 2006;28(3):442-6.
6. Musshoff F, Madea B. New trends in hair analysis and scientific demands on validation and technical implementation. *Forensic Sci Int*. 2007;165(2-3):204-15.
7. Chaturvedi AK, Canfield DV. Role of immunoassays in forensic toxicology. *J Anal Toxicol.* 1995;19(5):384-90.
8. Huestis MA, Verstraete AG, Kwong TC, Morland J, Vincent MJ. Oral fluid testing: promises and pitfalls. *Clin Chem*. 2011;57(6):805-9.
9. Moriya F, Hashimoto Y. Potential for error in forensic toxicological analysis due to postmortem drug redistribution. *Forensic Sci Int*. 1999;103(2):121-30.

DOI: 10.69605/ijlbpr_14.3.2025.204

10. Dinis-Oliveira RJ, Carvalho F, Duarte JA, Remião F, Marques A, Santos A, et al. Collection of biological samples in forensic toxicology. *Toxicol Mech Methods*. 2010;20(7):363-414.
11. Vogliardi S, Marchei E, Agostini C, et al. Simultaneous analysis of drugs of abuse and pharmaceuticals in oral fluid by LC-MS/MS. *J Chromatogr B*. 2006;844(2):314-21.
12. Drummer OH. Drug testing in oral fluid. *Clin Biochem Rev*. 2006;27(3):147-59.
13. Polettini A, editor. *Applications of LC-MS in toxicology: new frontiers in analytical toxicology*. Cambridge: RSC Publishing; 2006.
14. Boschen MJ, Slabbert JL. Advances in immunoassay technologies for forensic toxicology. *Bioanalysis*. 2017;9(18):1389-402.
15. Maurer HH. Systematic toxicological analysis of drugs and their metabolites by gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl*. 1992;580(1-2):3-41.