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Forensic Analysis of Herbicide Formulations Using GC-QQQ-MS and LC-QQQ-MS: A Comprehensive Review

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Abstract
Herbicides, which are commonly used in agriculture, present a major challenge in forensic toxicology because of their environmental contaminating potential and misuse in poisoning crimes. The present review considers the forensic examination of herbicides with special reference to the application of Gas Chromatography with Triple Quadrupole Mass Spectrometry (GC-QQQ-MS) and Liquid Chromatography with Triple Quadrupole Mass Spectrometry (LC-QQQ-MS) in the determination of common herbicides such as paraquat, atrazine, glyphosate, metribuzin, and oxyfluorfen. The said analytical techniques are very sensitive, specific, and can detect herbicides at trace levels in environmental and biological matrices. The article presents the chemical structure-based classification and mode of action of herbicides and highlights factors influencing the choice of detection strategies.
Keywords: Forensic toxicology, GC-QQQ-MS, LC-QQQ-MS, Pesticide detection, Herbicide formulation, environmental forensic.

1 Introduction

Herbicides are chemical compounds specifically formulated to minimize or eliminate unwanted weed production, in non agriculture and agricultural setting. The use of herbicide globally is tied directly to the demand for maximization of crop yields and better land management. However beyond their agricultural benefits, herbicides pose noticeable forensic and toxicological implications. There are lot of herbicides that are persistent in the ecosystem and too much capable of bioaccumulation, and it potentially leading towards severe health effects in humans as well as animals wildlife. There are many compounds link with acute poisoning, intentional ingestions in suicides, contamination of water supplies and food that necessitate forensic investigation (Vishaka et al., 2023).

The forensic examination of herbicides formulation has booming as a crucial area within environmental forensic and forensic toxicology. The exact quantification and identification of herbicides in biological fluids, water, plant matter and soils are crucial in cases involving occupational exposure, suspicious death and violations of pesticides regulation. Traditional analysis methods such as immunoassays and calorimatetry have limitations in sensitivity and specificity. Hence modern instrumental techniques like Liquid Chromatography-Triple Quadrupole Mass spectrometry (LC-QQQ-MS) and Gas Chromatography-Triple Quadrupole Mass Spectrometry (GC-QQQ-MS) are mostly used due to their advanced selectivity, low

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detection limits and high throughput (Syrgabek & Alimzhanova, 2022).
The review aims to investigate benefits of LC-QQQ-MS & GC-QQQ-MS in the forensic determination of herbicide formulations. Generally focus on common used herbicides – oxyfluorfen, glyphosate, metribuzin, paraquat & atrazine observing their forensic relevance, sample preparation, toxicokinetics, instrumental parameters and classifications. This review also highlights emerging research direction, analytical challenges and regulatory considerations.

2 Literature review

The quantification and detection of herbicides in different matrices have been significantly studied over the past two decades. The diversity of herbicides, especially organophosphorous derivatives (like glyphosate), bipyridyl compounds (like paraquat), triazines (like atrazines), have gained attentions because of their frequent presence in poisoning cases (Lovejoy & Fiumera, 2019).
Paraquat, for example, gives instant result because their high toxicity, mostly through ingestions. There are lot of case study report of intentional use for suicides, particularly in agro based community of South America and Asia. Zhang et al. (2020) reveal that LC-QQQ-MS methods allows the assessment of paraquat in biological fluids mostly in blood at amount low as 1 ng/mL, allowing for historical cohort confirmation of poisoning in forensic and clinical setting (Reihani et al., 2024).
Triazines derivatives herbicides such as atrazine, metribuzin, generally moderately toxic but they are persistent in groundwater and soil. Detection of atrazine in evironmetal forensic is crucial in monitoring groundwater contamination or in illegal agriculture. The recently study to put into GC-QQQ-MS for concurrent determination of atrazine with other triazine derivatives in water, getting high specificity and sensitivity (Jablonowski et al., 2010).
Glyphosate, generally regarded as less acutely toxic, frequently found in accidental exposure cases, because of its widespread use. It's non-volatile and polar in nature makes LC-QQQ-MS suitable method for detection. However derivatization is mostly required prior to LC/MS/MS analysis. The latest advancement has allowed direct injection method, boosting analytical efficiency (Taylor et al., 2022).
Oxyfluorfen and metribuzin are latest additions into the forensic herbicide profile; there are some which is indicating their detection in surface water and food residues. There is a report for the successful application of QuEChERS-based extraction coupled with LC-QQQ-MS for the detecting these herbicide traces at sub-ppb levels in vegetable (Chen et al., 2022).Continuous research have frequently highlighted the importance of combining efficient sample preparation with latest MS detection technique to control matrix disturbances. The invention of QQQ-MS has drastically improved the sensitivity and selectivity of herbicide determination, enabling detection of trace level amount & complex matrices (Zhe et al., 2020).

3 Classifications of Herbicides

Generally herbicides can be identifying on different different criteria such as selectivity, translocation behavior, time of application, mode of action, chemical structure. This classification is significant in forensic science, as it helps in determining the likely exposure routes, detection strategies & persistence in environmental and biological samples (Bcpc, n.d.)
Table no 1 Classification of herbicides based on their chemical structure

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Chemical class	Example herbicides	Key characteristics
Bipyridyls	Paraquat, diquat	High polarity, photoreactive, very toxic
Triazines	Atrazine, simazine	Moderately toxic, persistent in groundwater
Orgaophosphonates	Glyphosate	Polar, non-volatile, often requires derivatization
Triazinones	Metribuzin	Systemic, moderate environmental persistence
Diphenylethers	Oxyfluorfen	Pre-emergent, photodegradable

Data from (Plant Toxicology, 2004)

Herbicides can also be paired by their biochemical effect on target site. This classification is crucial to understanding toxicity profiles and symptoms of exposure in forensic cases (Kumar et al., 2023).

Table no 2 herbicide classification based on MoA

Mode of action	Herbicides	Action mechanism
Photosystem l inhibitors	Paraquat	Generates reactive oxygen species, causing cell damage
Photosystem ll inhibitors	Atrazine, metribuzi	Inhibits electron transport in photosynthesis
EPSP synthase inhibitors	Glyphosate	Inhibits aromatic amino acid biosynthesis
PPO (photoporphyriogen oxidase) inhibitors	Oxyfluorfen	Disrupts chlorophyll biosynthesis

Data from (*Weed Management Handbook*, n.d.)

This type of differentiation not only maximize forensic interpretation about poisoning symptoms but also influence sample preparation methodology, since chemical reactivity can be an obstruction in extraction and degradation (Malik & Baghel, 2023)

4 Analytical Techniques for Herbicide Detection in forensics

For the identification and quantifications of herbicides, instrumental techniques plays a very pivotal role particularly in different matrices such as food, blood, urine, soil. The most advance and effective approaches involve mass spectrometry because of its selectivity, sensitivity and provide structural information (Ohara et al., 2021).

4.1 Gas Chromatography with Triple Quadrupole Mass Spectrometry (GC-QQQ-MS)

GC-QQQ-MS generally used for semi-volatile or volatile herbicides, prominently those that are thermally stable or can be derivatized. QQQ configurations gives higher sensitivity and allows for multiple reaction monitoring (MRM), decrease interference from co-extracted matrix compounds. For instances, oxyfluorfen and atrazine are efficiently quantified and separated using GC-QQQ-MS. Demonstrated low limits of detection in environmental water sample using GC-MS/MS in MRM mode. GC system frequently incorporate automated derivatization or sample concentration units to enhance performance (Hernández-Mesa & Moreno-González, 2022).

However, polar herbicides like paraquat and glyphosate are not amenable to direct GC analysis and needs transformation into volatile derivatives, which is bit complicated process and maximizes the risk of contamination or analyte loss (Taylor et al., 2022b).

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4.2 Liquid Chromatography with triple Quadrupole Mass Spectrometry (LC-QQQ-MS)

LC-QQQ-MS is a method of choice mostly for thermally labile, on-volatile, or highly polar herbicides such as metribuzin, paraquat, & glyphosate. It bypasses the need of derivatization, and its soft ionization technique allows for intact detection of ionic herbicides (Mishra et al., 2023).

LC-QQQ-MS has become a gold standard technique in forensic science and toxicology laboratories because of its ultra-trace detection facility. For example successfully used of LC-QQQ-MS for the analyzing paraquat present in plasma with an LOD of 0.2 ng/mL, even after long period of storage (AZoLifeSciences, 2025).

Latest advancement includes the use of QuEChERS extraction technique in co-existence with LC-QQQ-MS for analysis of herbicide residue present in soil and vegetables (Vicari et al., 2024).

Table no 3 recommendation of instrument for particular herbicides

Instrument	Preferred herbicide	LOD Range	Key advantages
GC-QQQ-MS	Atrazine, oxyfluorfen	0.0005-0.05 µg/L	High sensitivity for volatile analytes
LC-QQQ-MS	Paraquat, Glyphosate,metribuzin	0.0002-0.01 µg/L	Suitable for polar, no-volatile, ionic herbicides

Data adapted from (Schreiber & AB SCIEX, 2013; Xu et al. (2022b)

5 Biological Samples (blood, Urine, Tissues)

Biological fluids are the primary matrices to analyze in forensic toxicology for suspected poisoning. Because of polar nature of herbicide such as glyphosate and paraquat, solid-phase extraction (SPE), ion pair extraction, protein precipitation is commonly used.

- Paraquat: Paraquat extraction generally involves acidification followed by SPE using cation-exchage cartridges (Baeck et al., 2007).
- Glyphosate: because its high polar nature, derivatization with FMOC-CI is typically required before the determination of LC-MS/MS, but recent methods allow aqueous injection, bypassing the complex step (Fur et al., 2000).
- Residue of oxyfluorfen in vegetable: recently analyzed market sample for pesticides residue and found out oxyfluorfen in spinach using LC-QQQ-MS (Zhao et al., 2023).
- Atrazine in water contamination: It is investigated that illegal atrazine discharge in rural water bodies. GC-QQQ-MS detected the concentration of atrazine exceeding 0.3 µg/mL, above the EU safety norms (Lee et al., 2024).

These case studies highlighting the concern that in herbicide determination is mandatory, and point out the need for robust analytical and sensitive techniques.

6 Analytical Challenges and Future Challenges

Regardless of advances in GC-QQQ-MS & LC-QQQ-MS, a number of challenges remain:

6.1 Analytical Challenges (Mallet, 2014; Handford et al. (2015)

- Matrix effects: soil and biological samples frequently produce co-eluting compounds that can be interference ionizations in MS.
- Derivatization Needs: Compounds such as glyphosate frequently need derivatization for analysis of GC, and it is adding complexity and time.

- Stability: Herbicides degrades quickly unless preserved in a proper way, which can be a impacting in forensic reliability.
- Regulatory discrepancy: Different country has their permissible limits and detection limits, and it can be a complicating factor for international casework.

6.2 Emerging Trends (Mol et al., 2016; Kaur et al. (2021; (Pesticides Mass Spectral Library With LRI, 2nd Edition - Wiley Science Solutions, 2025)

- High-Resolution Mass Spectroscopy (HRMS): it offers accurate mass data & it helps in untargeted screening for unknown.
- Automated Sample Preparation: It helps in enhance the throughput reproducibility.
- Green Analytical Chemistry (GAC): Focusing on the reducing solvent use and waste in pesticide residue determination.
- Forensic Database: Expansion in the herbicides mass spectral libraries can streamline the compound identification.

7 Conclusions

Forensic analysis of herbicide formulation has gained an immense importance because of increasing the case of intentional and accidental poisoning, occupational exposure, and ecosystem contamination. This review paper point out the role of LC-QQQ-MS & GC-QQQ-MS as vital tools in quantifying and detecting herbicides such as metribuzin, oxyfluorfen, paraquat, glyphosate and atrazine.

GC-QQQ-MS is typically suited for semi-volatile and volatile compounds like oxyfluorfen and atrazine, offering selectivity and high sensitivity in complex matrices. On the different sides, LC-QQQ-MS prevails in detecting thermally unstable and polar compounds like glyphosate and paraquat, mostly in food samples and biological fluids. Advanced sample preparation techniques like QuEChERS and SPE have increased the reliability of forensic work flows.

Despite the fact that current methodologies and techniques are robust, but there are some challenges such as regulatory inconsistencies, matrix effect, derivatization requirements still persist. The future scope involves amalgamation of high-resolution mass spectroscopy, green analytical practices and automation practices into forensic herbicides analysis. Eventually as forensic and environmental concerns grow, analytical toxicologist should refining methodology to improve reliability, sensitivity, and legal defensibility in herbicide related investigation.

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