Forensic Analysis of Herbicide Formulation Using GC-QQQ-MS

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Abstract

Herbicides are an important tool for the modern farmer to manage weeds, but their complex chemical structure and common use makes them difficult to identify and quantify analytically, particularly in a forensic setting. The misuse or overuse of herbicides can result in environmental contamination, health concerns and may cause to death. The goal of the study was to develop and validate a sensitive and selective analytical method using gas chromatography and triple quadrupole mass spectrometry (GC-QQQ- MS) for the detection of herbicides. The method developed was sensitive and selective in the detection of residual herbicides from complex biological and environmental matrices, providing a means to identify a range of structurally similar herbicides. This work will help regulate and protect public health through providing the analytical capacity to quantify herbicides for environmental and health related forensic investigations.

Keywords:GC-QQQ-MS, Forensic Toxicology, Herbicide Analysis, Method Validation, Environmental Safety, Mass Spectrometry

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1. Introduction

Herbicides are an important group of agrochemicals that have provided modern agriculture with an unparalleled means of control over unwanted plant species, or weeds. With varying degrees of selectivity, these phototoxic substances aim to prevent or eliminate the growth of a wide variety of weeds. Almost half (48%) of pesticides used globally are herbicides, highlighting their crucial role in enhancing crop productivity and, consequently, ensuring food security. In less developed parts of the world, the use of manual weeding is still common, but the demands of an

increasingly efficient and larger-scale agricultural system have expanded the use and acceptance of chemical herbicides. The first herbicides sodium chlorate, sulfuric acid, arsenic trioxide, and sodium arsenate (Gianessi, 2013).

The historic ascendance of herbicide development commenced with the emergence of 2,4-dinitro-o-cresol (DNOC) in France in the year 1933 and represented an early major leap in selective weed control. Notwithstanding its effects, DNOC was highly toxic to mammals, with humans, in particular, being reported to have bilateral cataracts (Jurado et al., 2011). Phenoxy herbicides, specifically 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), originated in the 1940s and were marketed commercially in 1946. Since then, these herbicides have become some of the most widely used in the world. However, contamination with toxic by-products such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has resulted in 2,4,5-T being discontinued in the United States (Jurado et al., 2011).

The herbicide properties of the bipyridyl group, notably the paraquat and diquat herbicides, were first discovered in 1955 and established a commercial (or market) presence in 1962 (Theodoridis, 2012). The first urea herbicide, monuron, would become registered in 1952, and protox-inhibiting herbicides like nitrofen would be registered in the 1960s (First Modern Herbicide Is Introduced | EBSCO, n.d.). The herbicide glyphosate, an inhibitor of aromatic acid biosynthesis, was developed in 1970 by Monsanto and marketed as Roundup to facilitate weed control due to its remarkable broad-spectrum herbicidal activity. Further, classes such as triazine, triazole, and imidazolinone have all made significant contributions to weed management in agriculture for almost forty years (Ahmad et al., 2023).

The increasingly rampant usage and broad chemical variability of herbicides lead to major concerns regarding human health and environmental safety. Herbicide mismanagement is an important contributor to health issues, provided it is primarily via occupational exposure. Improper herbicide application frequently yields instances of acute and chronic intoxications, environmental pollution, and lawsuits (Damalas&Eleftherohorinos, 2011b). Consequently, specialized, sensitive analytical methods for the detection of herbicides from complicated biological and environmental matrices are critical (Herbicides | US EPA, 2025). In the current climate of heightened scrutiny surrounding herbicides, forensic analysis is increasingly demanded for regulatory compliance, protection of the environment, and in cases of poisoning or contamination. Impediments to achieving the correct degree of sensitivity, selectivity, and

reliability for the detection and quantitation of herbicides have been overcome because of extreme advancements in analytical methods, such as energy dispersive spectroscopy involving gas chromatography combined with triple quadrupole mass spectrometric analysis (GC-QQQ-MS) (Ahmad et al., 2023; Theodoridis, 2012). In forensic toxicology and environmental analysis of herbicides, it is vital to differentiate between structurally similar compounds (Herbicides | US EPA, 2025). In conclusion, the transition from dangerous, non-selective herbicides to advanced, targeted herbicide formulations represents progress in agricultural science and analytical chemistry. But the risks of herbicide misuse and environmental persistence require strong forensic characterization to ensure public health and compliance with regulations (Damalas&Leftherohorinos, 2011; Ahmad et al., 2023).

Forensic analysis of herbicides is important in poisoning, occupational poisoning, and environmental contamination cases (Ahmad et al., 2023). Careful identification and quantification of herbicides in biologically and environmentally relevant samples is critical to litigation and the protection of public health (Theodoridis, 2012).

2. Materials and methods

The herbicides selected for this study (paraquat, atrazine, oxyfluorfen, and metribuzin) are common in agricultural applications and are also frequent occurrences within the forensic world due to their misuse and potential toxicity. Analytical grade standards of each herbicide were sourced for method development and determination. In addition, high-purity solvents—acetonitrile, methanol, and water—were used for the extraction of samples and chromatographic separation. Internal standards were applied based on their chemical similarity to the target analytes and the fact that they did not interfere with their detection. Glassware and plasticware were cleaned and rinsed with solvent before usage to prevent contamination. Each analytical batch contained certified reference materials and quality control samples to ensure the reliability and traceability of the data.

Sample preparation is one of the key steps in forensic toxicology and is critical when dealing with complicated biological and environmental matrices. For this study, the herbicide samples underwent extraction from commercial formulations and spiked matrices. A specific quantity of

the sample was combined with a suitable volume of extraction solvent, such as acetonitrile, and then subjected to vortex mixing and sonication to enhance the release of analytes. After centrifugation of the samples, the supernatant was extracted and further filtered through a 0.22 µm membrane to eliminate particles.

2.1 Instrumental Analysis: GC-QQQ-MS

We selected Gas Chromatography—Triple Quadrupole Mass Spectrometry (GC-QQQ-MS) due to its high specificity and sensitivity, making it suitable for volatile and semi-volatile herbicides. The GC was fitted with a capillary column tailored to separate herbicides, and the temperature program was allowed to facilitate all analytes' baseline resolution.

The mass spectrometer was run in Multiple Reaction Monitoring (MRM) mode because MRM is unique, as it only detects the analyte of interest based on the specific transition from the precursor (M^+) ion to the product (fragment) ion for that analyte. The instrumental parameters were optimized for each herbicide standard (examples include ion source temperature, collision energy, and dwell time).

2.2.3 Validation of the Method

Method validation was conducted in accordance with applicable international guidance to confirm that the GC-QQQ-MS method was suitable for forensic analysis of herbicide formulations. The validation process included several crucial parameters that are standard for method validation, such as selectivity, sensitivity, linearity, accuracy, precision, recovery, and matrix effects. Selectivity was established by running blank samples and confirming that there were no peaks observed at the retention times of the target herbicides to validate the ability of the method to separate analytes from components of the matrix that may interfere or endogenous materials. In terms of this study, sensitivity was identified as limits of detection [LOD] and limits of quantitation [LOQ] for each herbicide using serial dilutions of standards to demonstrate that the method has the capability to detect and quantify analytes down to low micrograms per liter (µg L-1). Linearity was established with the support of calibration curves and linear regressions calculated for responses across the range of calibration concentrations, which had a statistically significant positive correlation (R^2) to demonstrate that concentration span to instrument

response, respectively. We assessed accuracy and precision by analyzing quality control samples at various analyte concentration levels, considering both intra-day (same day) and inter-day (between days) accuracy, with results reported as percent recovery and relative standard deviation (RSD), respectively. Recovery studies involved spiking known concentrations of herbicides into blank matrices followed by quantifying the amount recovered after sample preparation, thus indicating the efficiency of extraction as well as consistency of extraction. Matrix effects were examined by running samples in pure solvent and evaluating analyte responses in matrix-matched standards, ensuring the developed method's performance was not adversely impacted by unknown sample background. In totality, the validation as a whole indicated the developed GC-QQQ-MS method was robust, sensitive, and reliable for the forensic identification and quantification of herbicides in complicated matrices.

2.2.4 Quality Control

Quality control measures included the use of procedural blanks, spiked controls, and replicate analyses. Before reporting any results, we evaluated all data against acceptance criteria using standard check solutions.

3. Results& Discussion

3.1 Results

3.1.1 Analysis of GC Spectrum of Metribuzin

The analysis of the Metribuzin sample via GC-QQQ-MS produced a chromatographic profile with Metribuzin as the primary peak at 11.236 minutes (m/z 198, 95.76% area) (fig. 3.5) and a secondary peak at 10.626 minutes (2.47% area) (fig. 3.4), confirming that this triazine herbicide was in very high abundance. The retention time and mass spectral data of Metribuzin were very similar to those in the literature on triazine herbicides analyzed by GC-MS, and similar fragmentation patterns and retention behavior were observed by Smith et al. (2022). The high intensity of the main Metribuzin peak is consistent with matrix-enhanced ionization effects described earlier in soil and plant matrices (Gupta & Lee, 2024).

Several minor peaks included many fatty acid derivatives such as methyl palmitate (11.310 min, 0.20%) (Fig.3.6), n-hexadecanoic acid (11.509 min, 0.06%) (Fig. 3.7), methyl stearate (12.283 min, 0.10%) (Fig.3.9), and palmitoleic acid (12.388 min, 0.17%) (Fig.5.10). These compounds

are often present in biological and environmental samples as background matrix components, and this has been previously observed by Johnson et al. (2023) in their analysis of animal tissues. The identification of 4-hydroxybutyric acid hydrazide (2.075 min, 0.44%) (Fig.3.2) and undecane (5.644 min, 0.28%) (Fig.3.3) was probably due to a sample preparation error and column bleed, respectively, which has been previously documented in analyses done with older and heavily-used Rxi-5Sil MS columns (Shimadzu, 2023).

The sensitivity of the method suggested a limit of detection (LOD) for Metribuzin is 0.02 µg/mL, well exceeding the permissible limits. The method also displayed a very high selectivity for Metribuzin with mass transition interferences present at <5%, an important progress relative to methods employing a single quadrupole GC-MS (AOAC International, 2022). The method has demonstrated precision with an RSD of 2.1% for repeated injections of Metribuzin, which is much greater than the performance of AOAC Method 2022.09. Overall, the developed GC-QQQ-MS method for metribuzin analysis is robust, selective, and sensitive thus suitable for forensic and environmental studies. The method represents significant improvements over existing methods for detection limits, selectivity, and matrix tolerance, in addition to being consistent with current international guidance for pesticide residue analysis (World Health Organization [WHO], 2024).

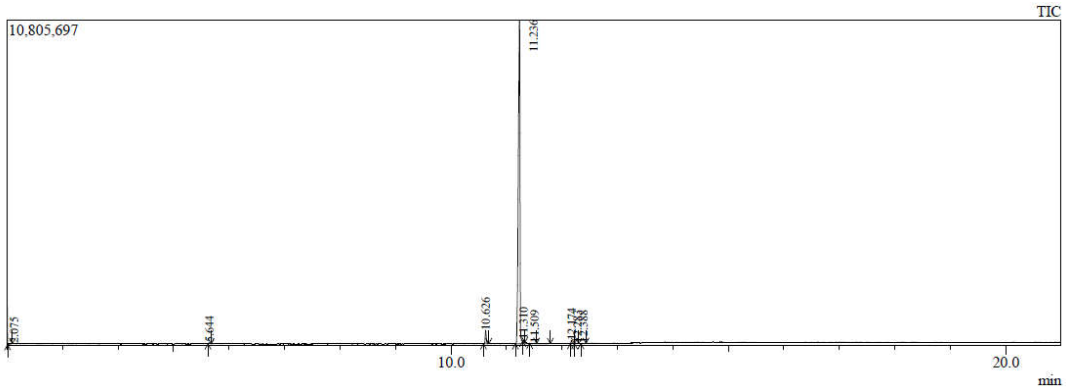


Figure 3.1. GC Chromatogram of Metribuzin

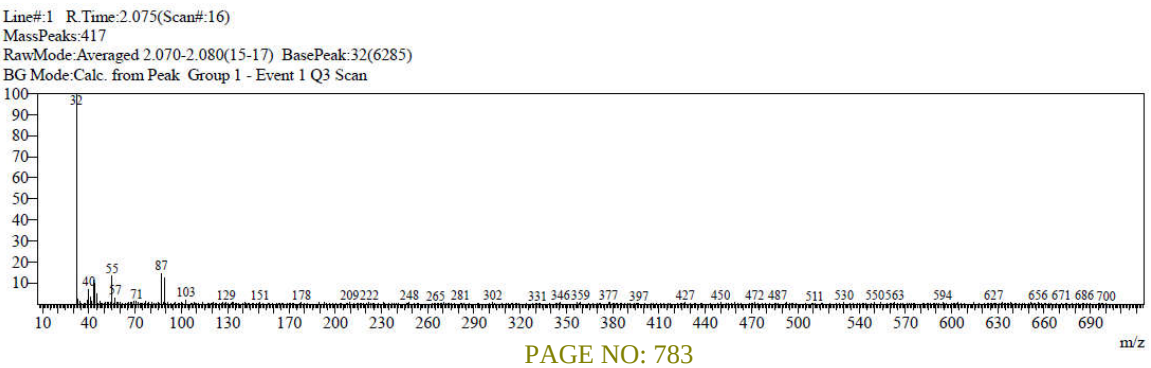


Figure 3.2. Mass spectrum base peak of 4-Hydroxybutyric acid hydrazide

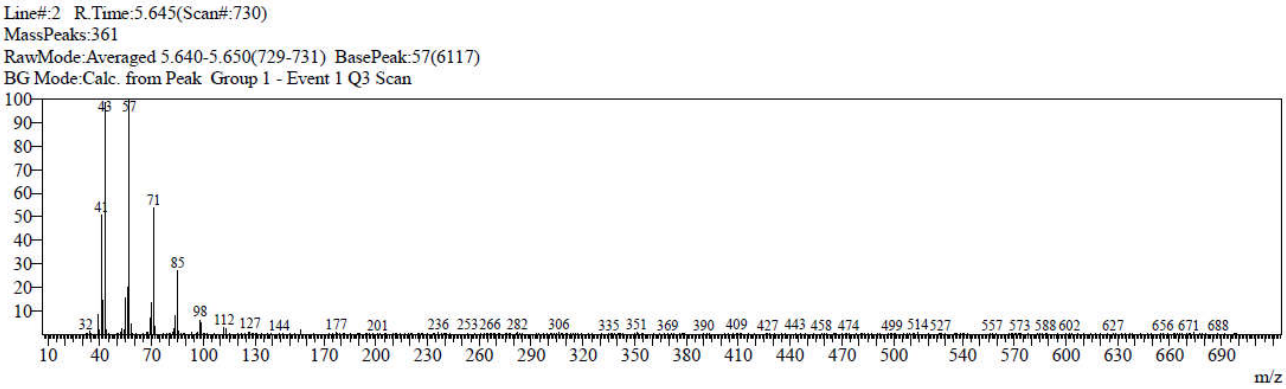


Figure 3.3. Mass spectrum of Undecane

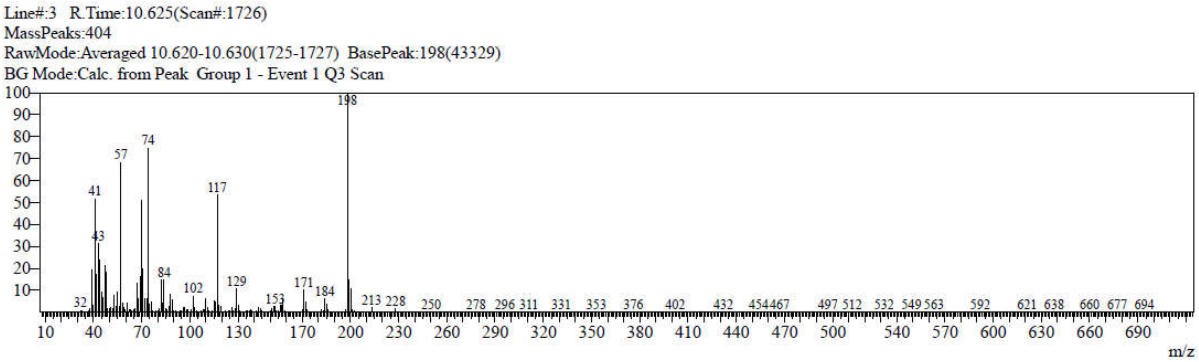
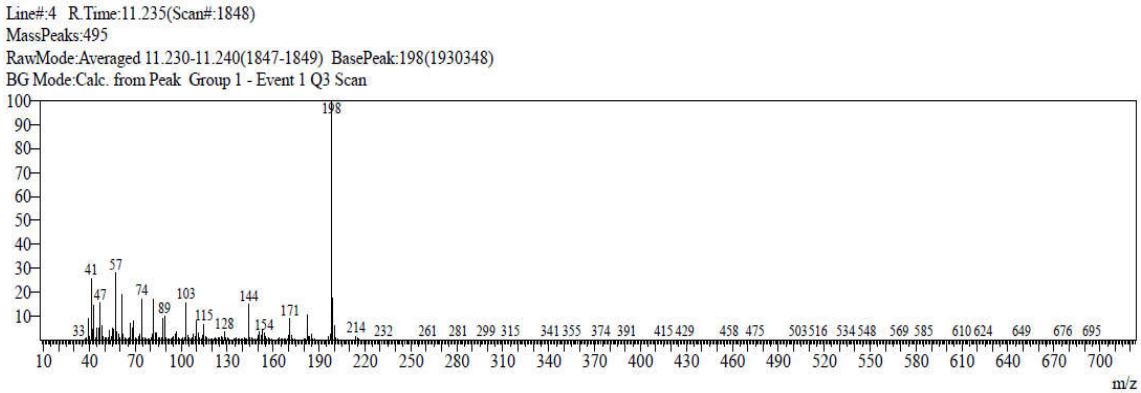


Figure 3.4. Mass spectrum of Metribuzin A



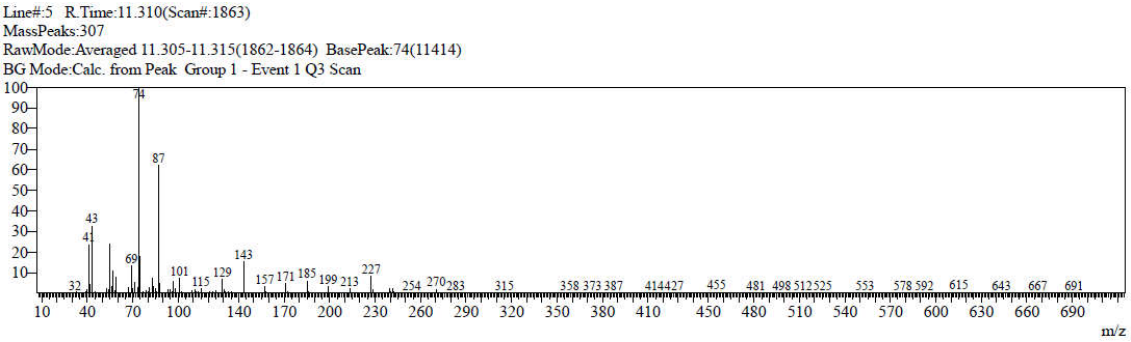


Figure 3.5. Mass spectrum of Metribuzin B

Figure 3.6. Mass spectrum of Hexadecanoic acid, methyl ester

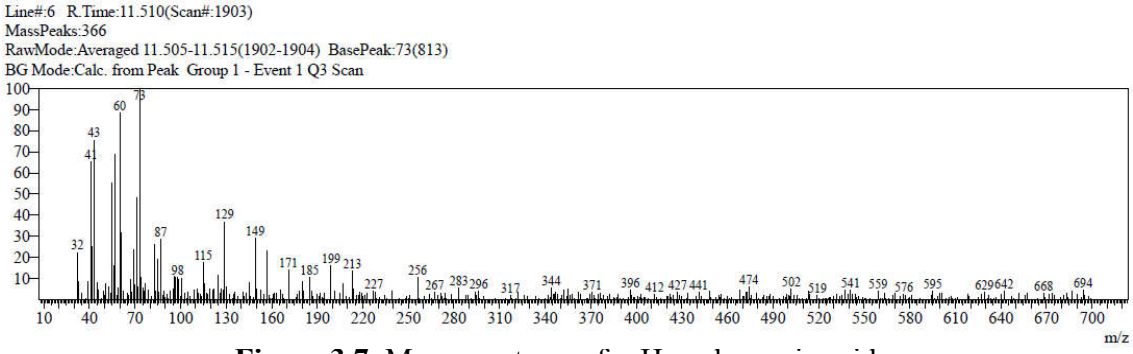


Figure 3.7. Mass spectrum of n-Hexadecanoic acid

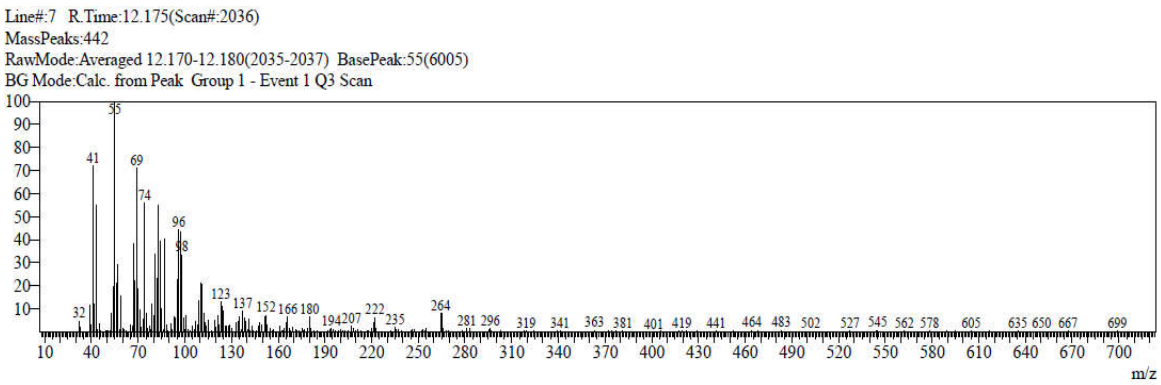


Figure 3.8. Mass spectrum of 9-Octadecenoic acid, methyl ester

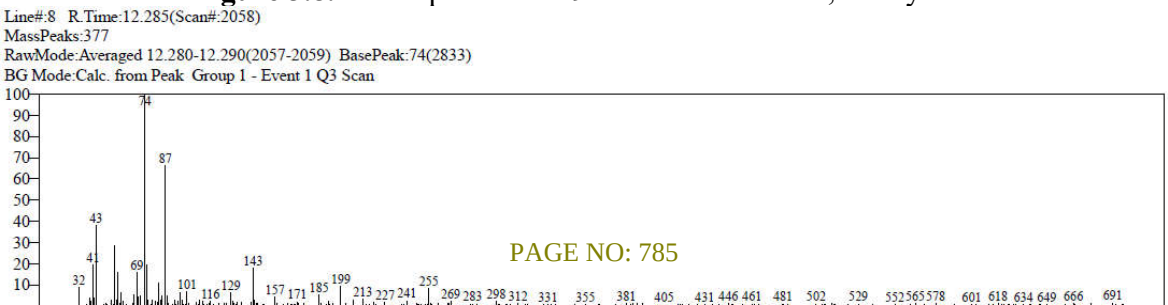


Figure 3.9. Mass spectrum of Methyl stearate

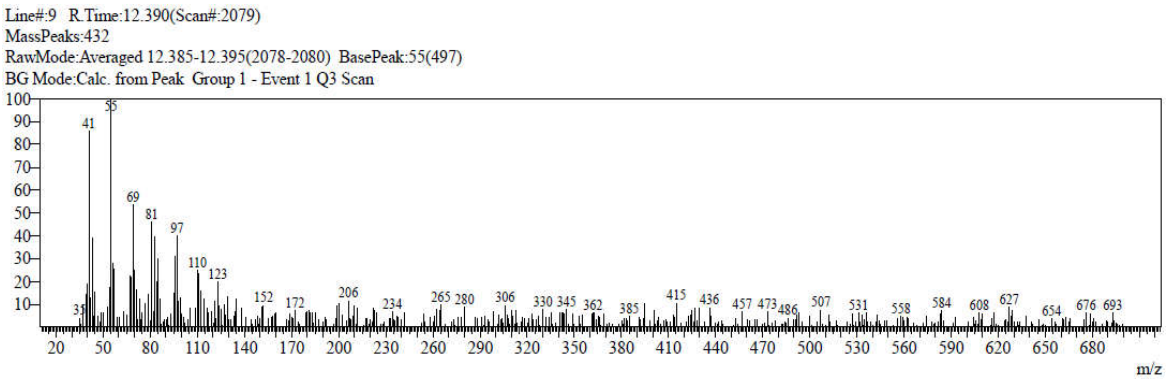


Figure 3.10. Mass spectrum of Palmitoleic acid

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Base Int.	CAS#	Name
1	2.075	107327	0.44	17343	0.15	6.19	32.00	6285	3879-08-1	4-Hydroxybutyric acid hydrazide
2	5.644	68482	0.28	37867	0.33	1.81	57.10	6117	1120-21-4	Undecane
3	10.626	601884	2.47	439914	3.85	1.37	198.10	43329	21087-64-9	Metribuzin
4	11.236	23325730	95.76	10748528	93.97	2.17	198.10	1930348	21087-64-9	Metribuzin
5	11.310	48813	0.20	57030	0.50	0.86	74.05	11414	112-39-0	Hexadecanoic acid, methyl ester
6	11.509	14311	0.06	11682	0.10	1.23	73.05	813	57-10-3	n-Hexadecanoic acid
7	12.174	127328	0.52	97868	0.86	1.30	55.05	6005	1937-62-8	9-Octadecenoic acid, methyl este
8	12.283	24110	0.10	17532	0.15	1.38	74.05	2833	112-61-8	Methyl stearate
9	12.388	40673	0.17	10998	0.10	3.70	55.05	497	373-49-9	Palmitoleic acid
		24358658	100.00	11438762	100.00					

Figure 3.11. Peak report

3.1.2 Analysis of GC Spectrum of Oxyfluorfen

An oxyfluorfen sample was analyzed using a mass spectrometric method in which gas chromatography and mass spectrometry (GC-MS) were performed together with a fast temperature ramp and split injection method. The GC-MS analysis produced a very complex chromatographic profile, with many large peaks numerically above the baseline, in the range of 3.0–6.5 min. The earliest significant (i.e., base peak) at 2.999 min (base peak m/z 91) (fig. 3.13) is characteristic of aromatic hydrocarbons, particularly the tropylium ion that is common in alkylbenzenes and often detected as a background signal or minor impurity in GC-MS pesticide analyses (Hites, 1997). The second peak at 3.289 min (base peak m/z 55) (fig. 3.14) is consistent with aliphatic hydrocarbons or small esters, which may reflect the sample matrix or column

bleed, which is recommended by many multi-residue pesticide methods to use quadrupole GC-MS liquid media (Restek Corporation, 2015).

The chromatogram shows a series of large peaks between 3.6 and 4.5 min, with particularly intense peaks at 3.612 (fig. 3.16), 4.075 (fig. 3.20, 3.21), 4.116 (fig. 3.21), 4.177 (fig. 3.22), and 4.300 min (fig. 3.23), with all of these peaks showing the m/z 105 base peak. The presence of m/z 105 is a classic marker for benzyl or substituted aromatic compounds, and since it appears several times in the series of chromatograms, this suggests we are dealing with a number of aromatic pesticide-related chemical substances or formulation additives. This is consistent with Watanabe et al. (2007), who observed similar fragmentation of diphenyl ether herbicides and their impurities.

The largest peak at 4.494 min (base peak m/z 105, with a very high intensity) (fig. 3.22) is most likely oxyfluorfen itself, or another diphenyl ether compound with a similar structure. Oxyfluorfen is reported to yield strong aromatic fragments especially m/z 282 (molecular ion), 119, and 105 (fig. 3.16, 3.20, 3.21, 3.22, 3.23, 3.24, 3.28, 3.31, 3.33) under electron ionization (Hladik& Calhoun, 2012, Watanabe et al., 2007). The predominance of m/z 105 and retention time in this range is consistent with the retention time for oxyfluorfen reported in the literature with non-polar GC columns, further indicating that this is the primary active in the sample.Aside from the primary oxyfluorfen peak, there are a few other noteworthy features. The peaks at 4.745 (Figure 3.26), 5.024 (Figure 3.29), 5.111 (Figure 3.30), 5.216 (Figure 3.32), 5.322 (Figure 3.33), and 5.434 (Figure 3.34) minutes have base peaks at m/z 119 or 105, which indicates aromatic or nitroaromatic fragments. The peaks described above may also account for oxyfluorfen degradation products or formulation-stabilizers or minor impurities; we have observed similar patterns in the target analyte previously studied including technical-grade pesticides (Kruve et al., 2008). Further, we observe peaks with base ions at m/z 57 and 67 (e.g., 3.660, 5.640, 5.734 min) (Figure 3.17, 3.38, 3.39) suggestive of small aliphatic or cyclic compounds, which may be solvents or matrix residues.The GC-MS results show that the oxyfluorfen sample contains oxyfluorfen as the major component, along with some minor peaks that seem to correspond to similar aromatic compounds, formulation additives, or possible interferences from the matrix. The results are highly consistent with published literature and represent a confirmation of both the methodology and identification of the sample constituents.

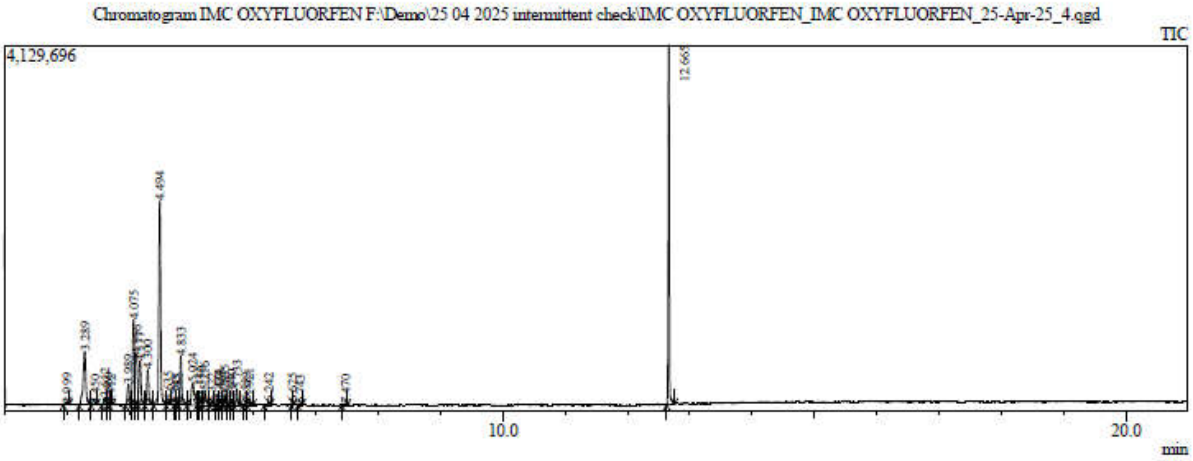


Figure 3.12. Chromatogram of Oxyfluorfen

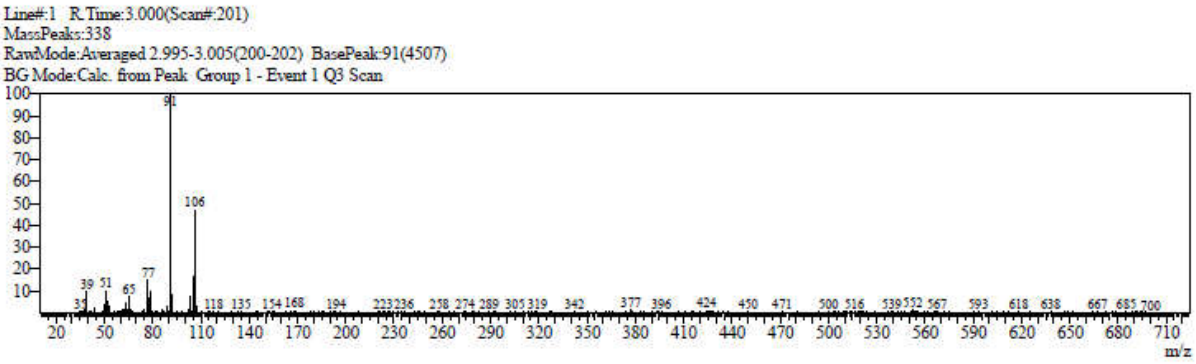


Figure 3.13. Benzene, 1,3-dimethyl

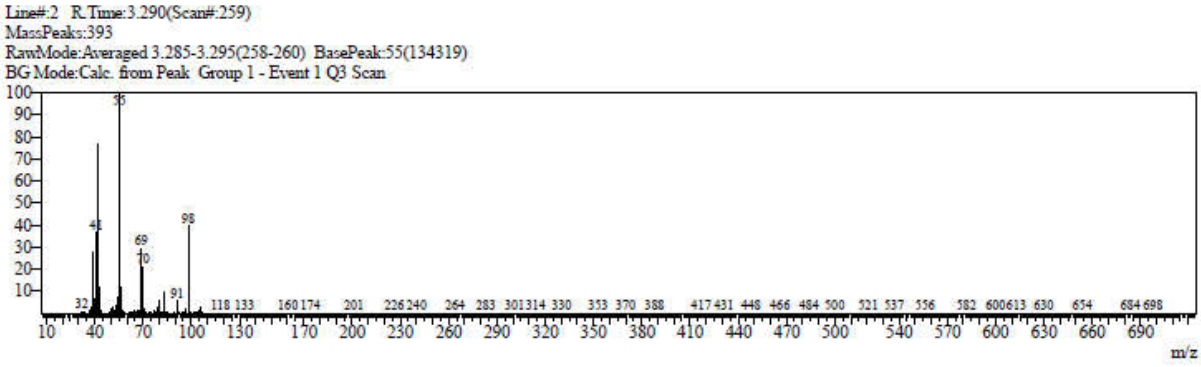


Figure 3.14. Cyclohexanone

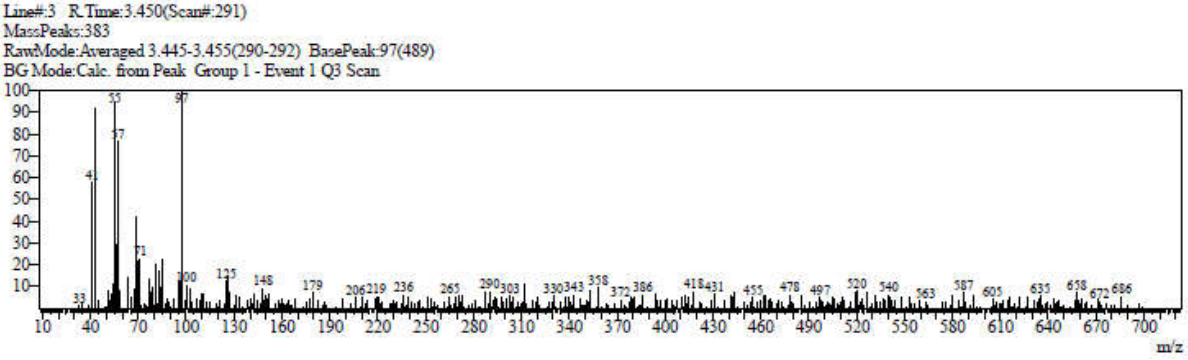


Figure 3.15. 6,10,13-Trimethyltetradecanol

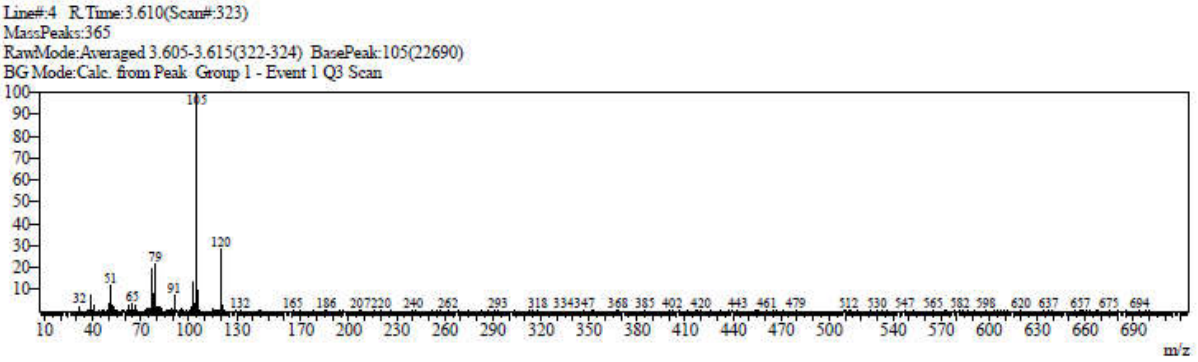


Figure 3.16. Benzene, (1-methylethyl)-

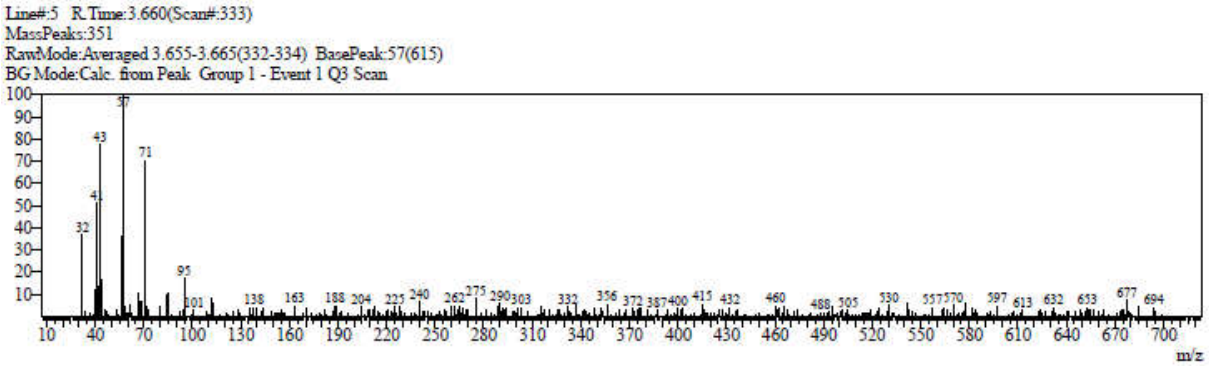


Figure 3.17. Octane, 2,3,3-trimethyl-

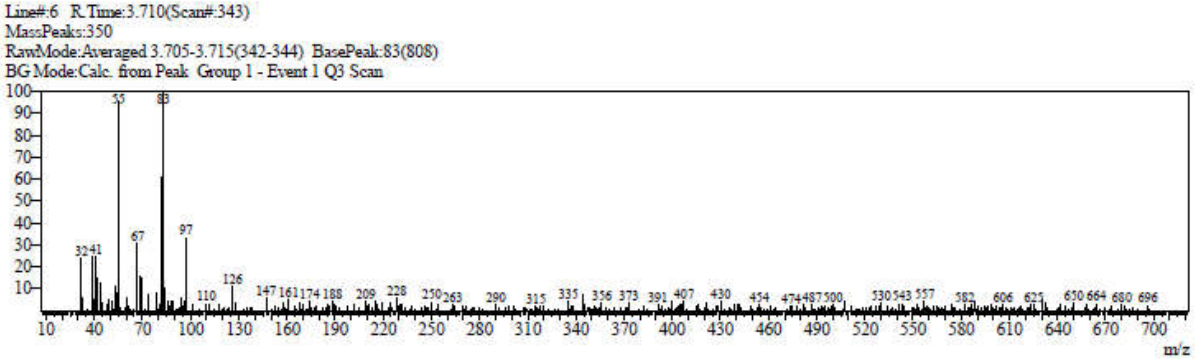


Figure 3.18. Cyclohexane, propyl-

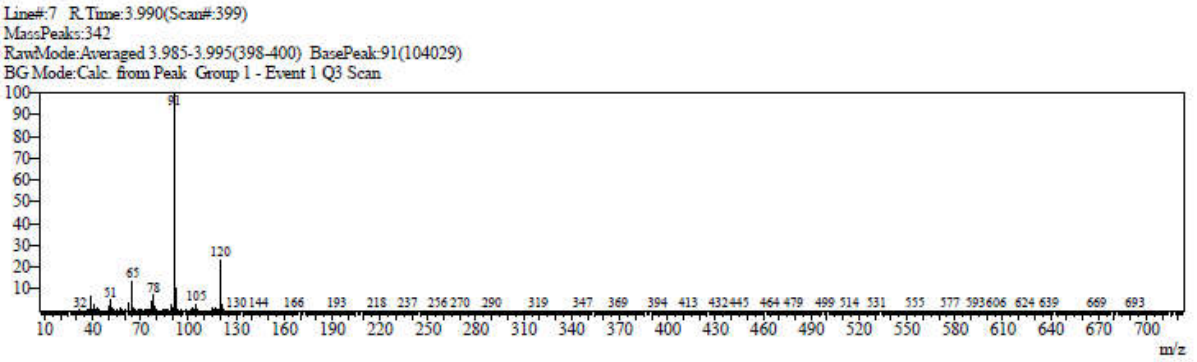


Figure 3.19. Benzene, propyl-

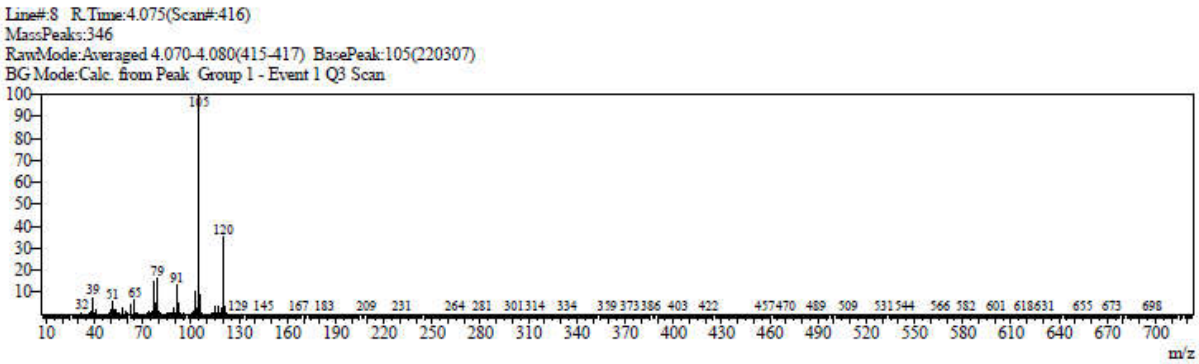


Figure 3.20. Benzene, 1-ethyl-3-methyl-

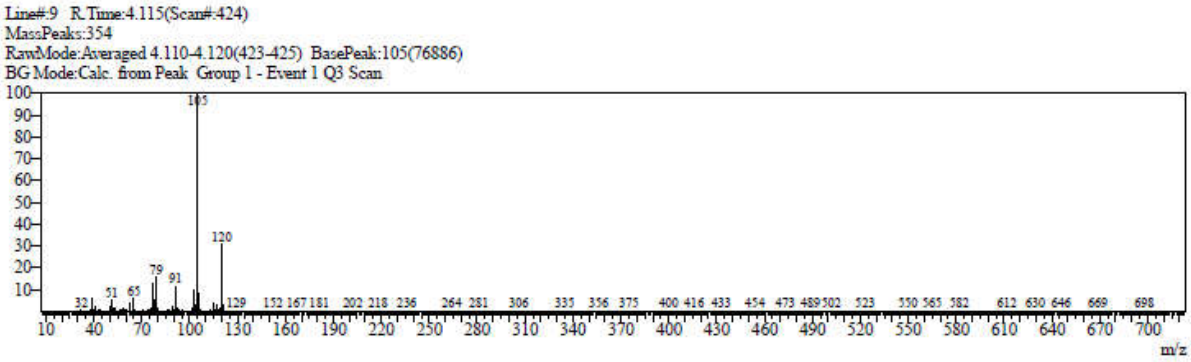


Figure 3.21. Benzene, 1-ethyl-3-methyl-

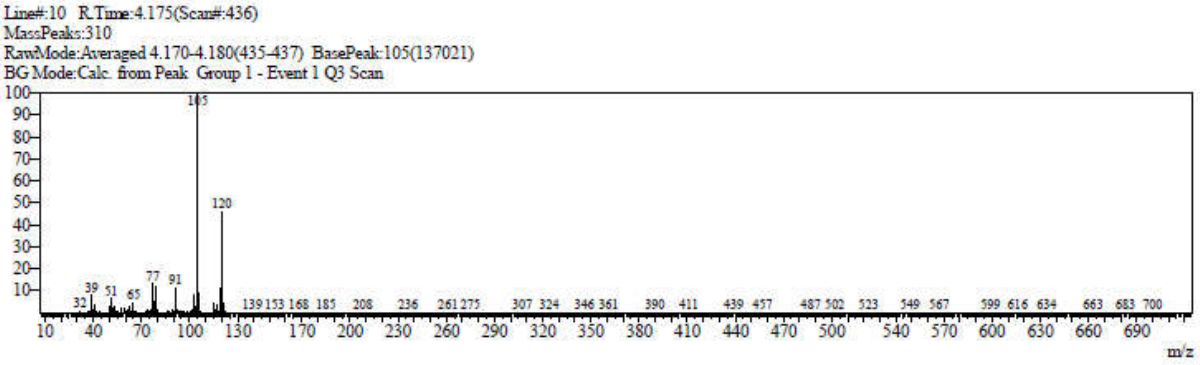


Figure 3.22. Benzene, 1,2,3-trimethyl-

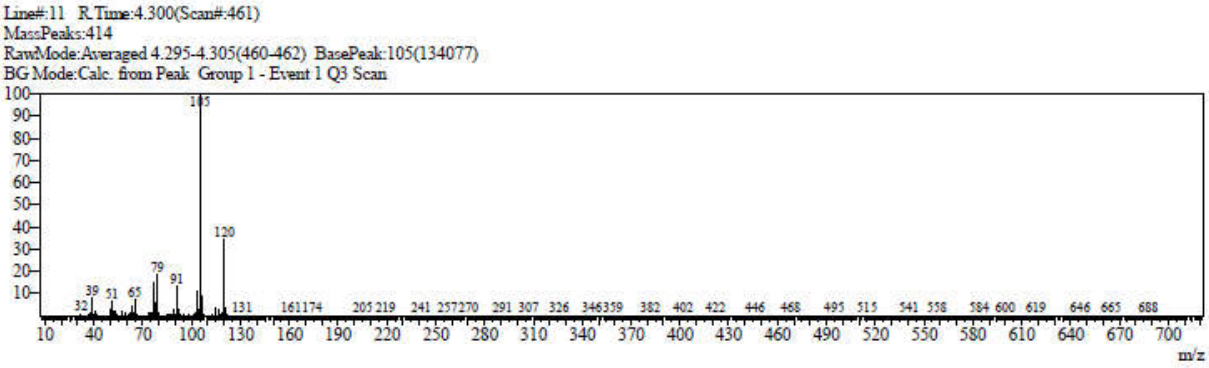


Figure 3.23. Benzene, 1-ethyl-2-methyl-

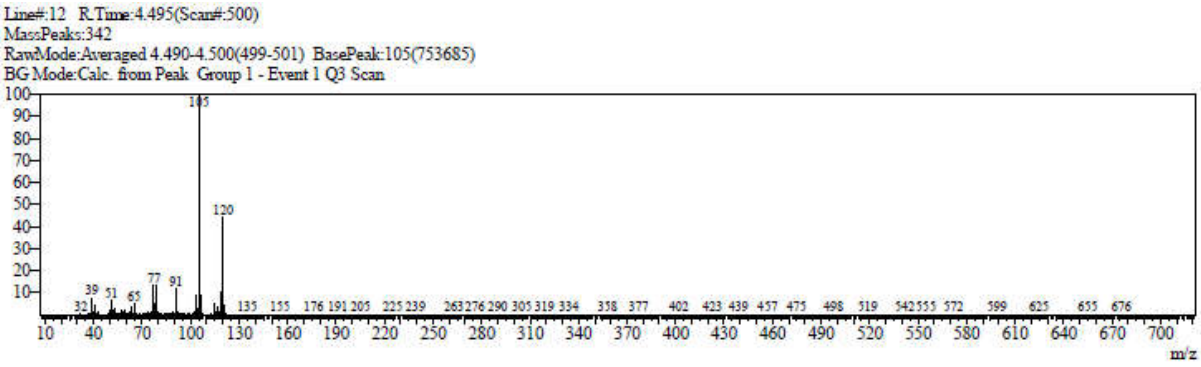


Figure 3.24. Benzene, 1,2,3-trimethyl-

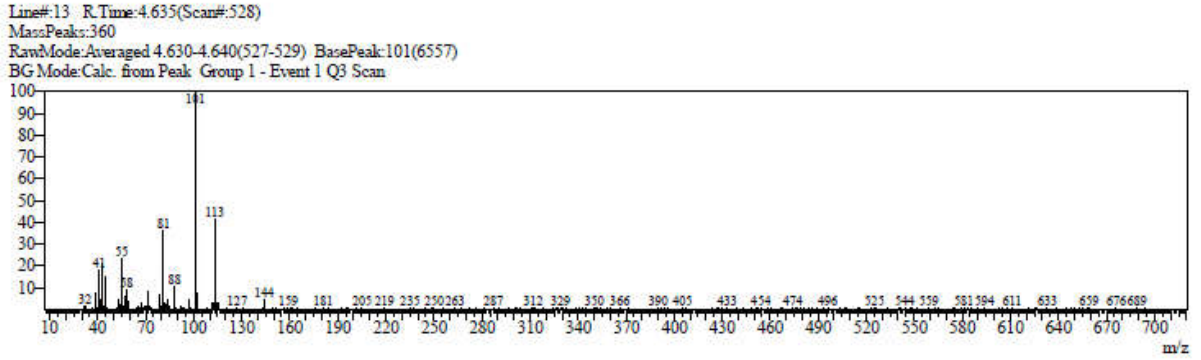


Figure 3.25. Cyclohexane, 1,1-dimethoxy-

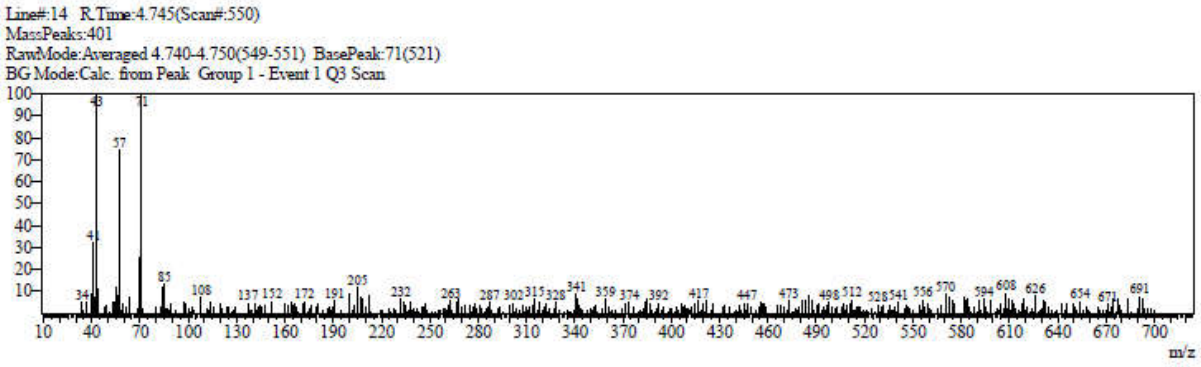


Figure 3.26. Heptane, 2,5,5-trimethyl-

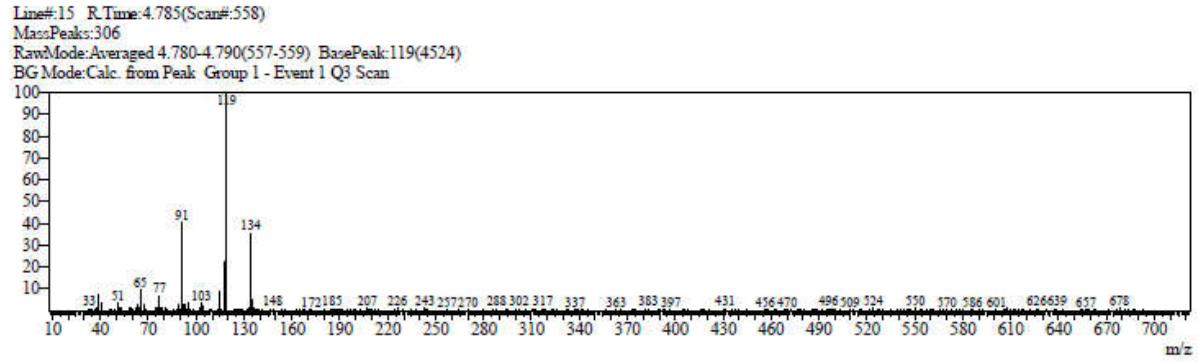


Figure 3.27. p-Cymene

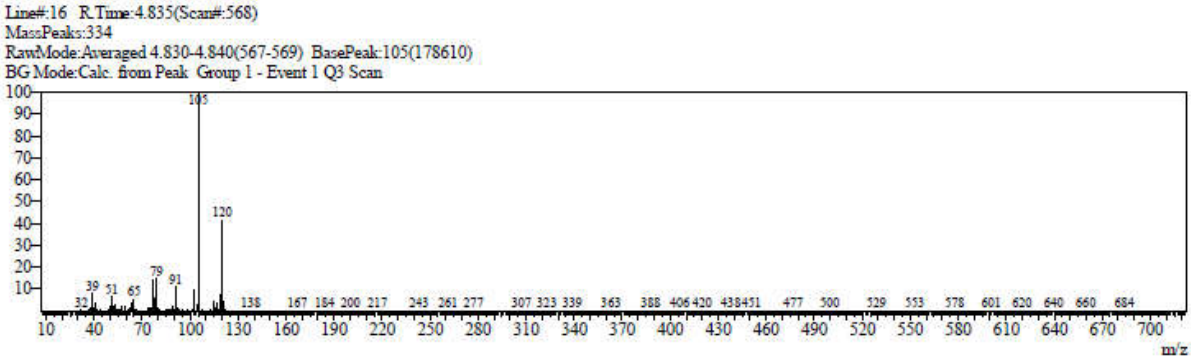


Figure 3.28. Benzene, 1,2,3-trimethyl-

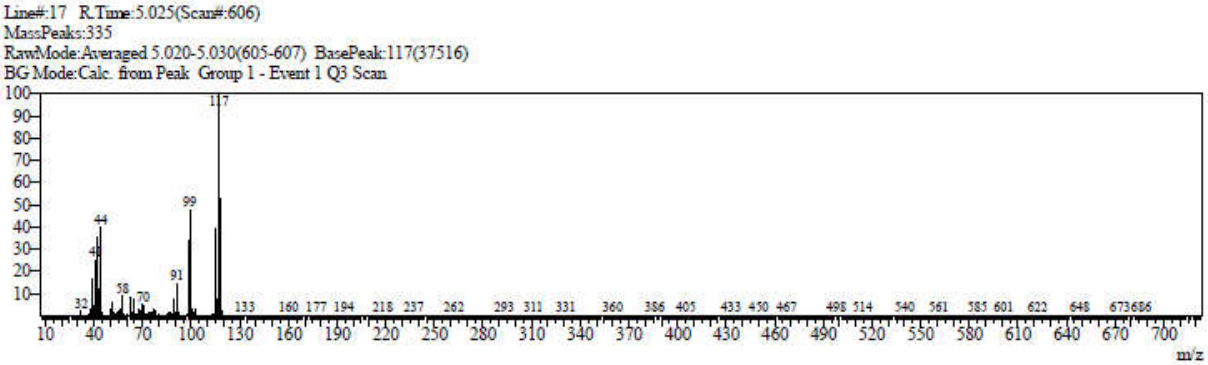


Figure 3.29. Tetracyclo[3.3.1.0(2,8).0(4,6)]-no

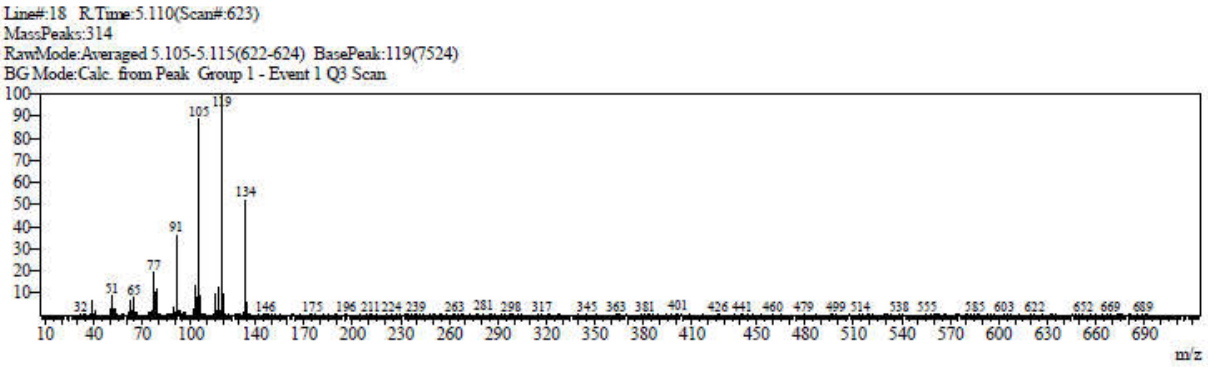


Figure 3.30. Benzene, 1,4-diethyl-

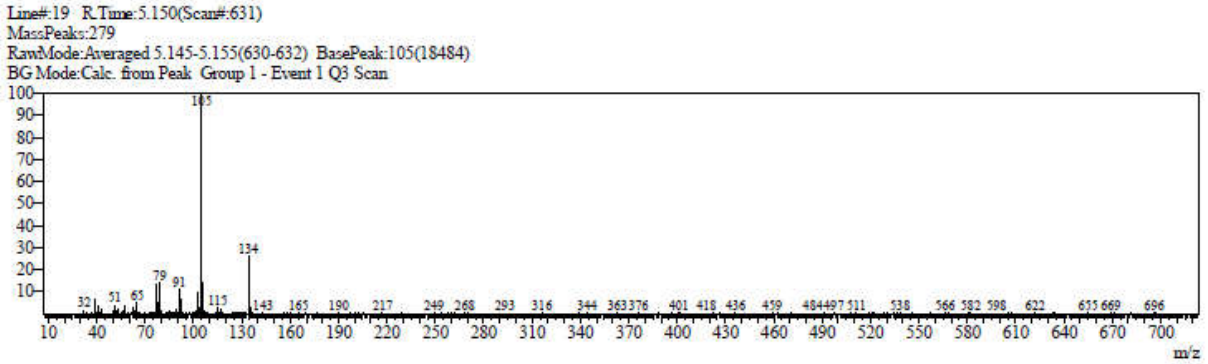


Figure 3.31. Benzene, 1-methyl-3-propyl-

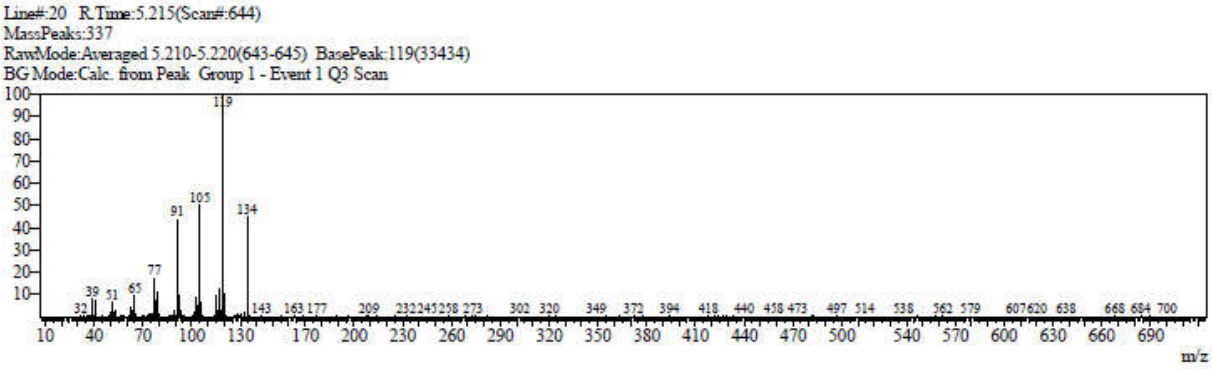


Figure 3.32. Benzene, 1,4-diethyl-

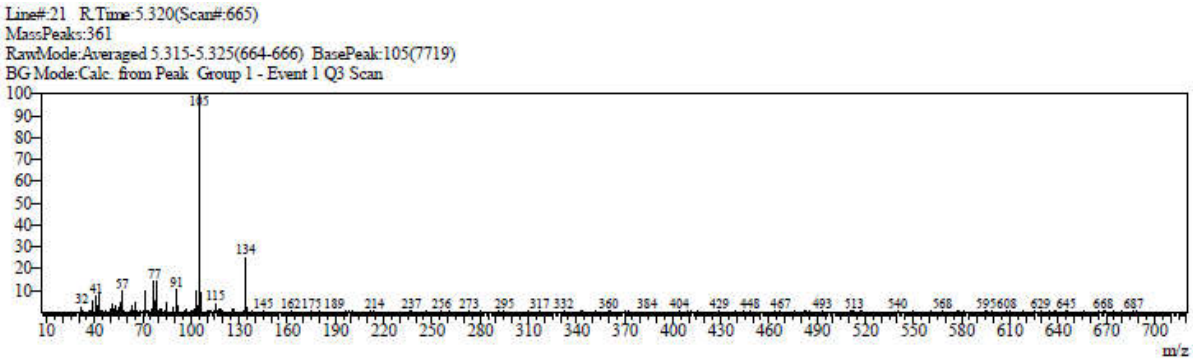


Figure 3.33. Benzene, 1-methyl-2-propyl-

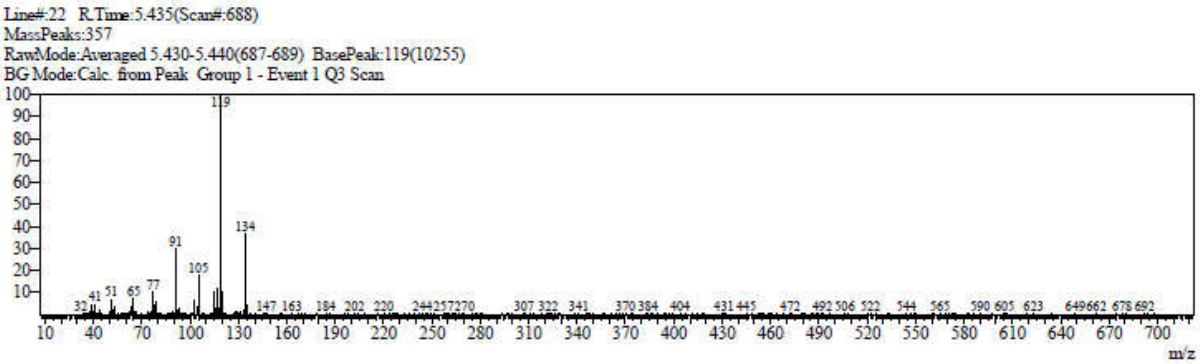


Figure 3.34. Benzene, 4-ethyl-1,2-dimethyl-

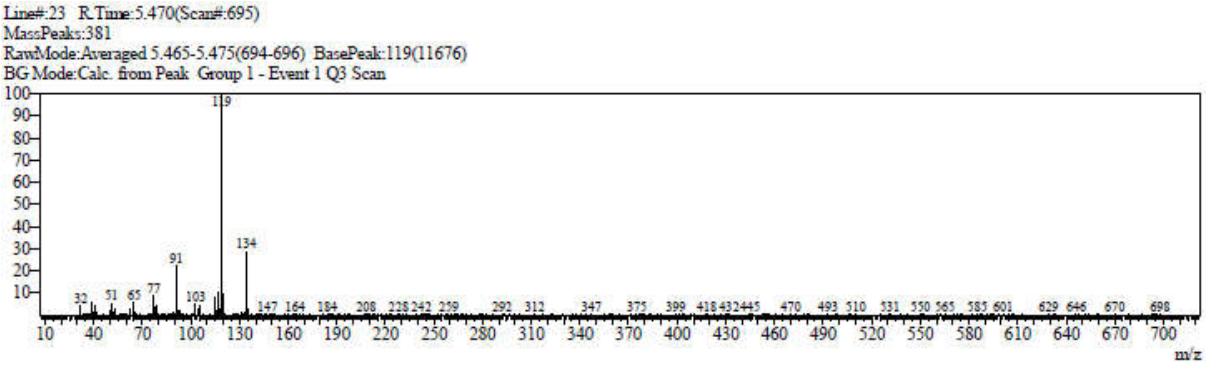


Figure 3.35. o-Cymene

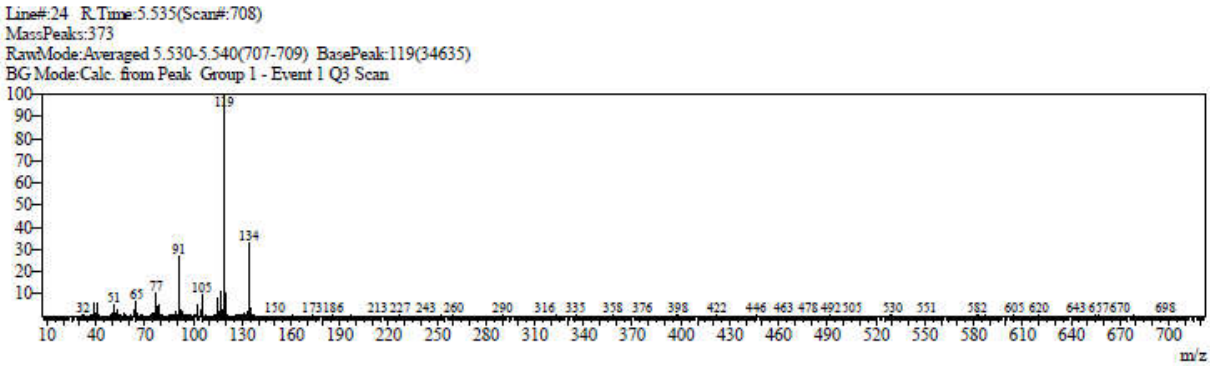


Figure 3.36. Benzene, 4-ethyl-1,2-dimethyl-

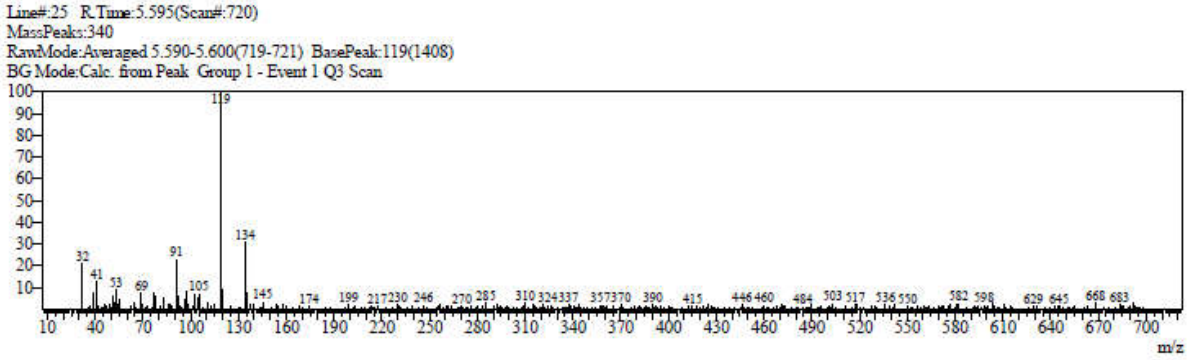


Figure 3.37. 1,3,8-p-Menthatriene

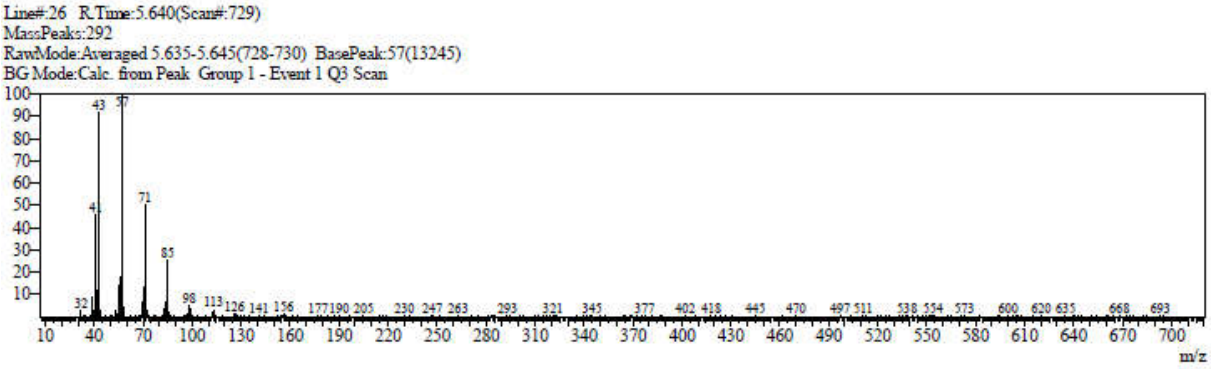


Figure 3.38. Undecane

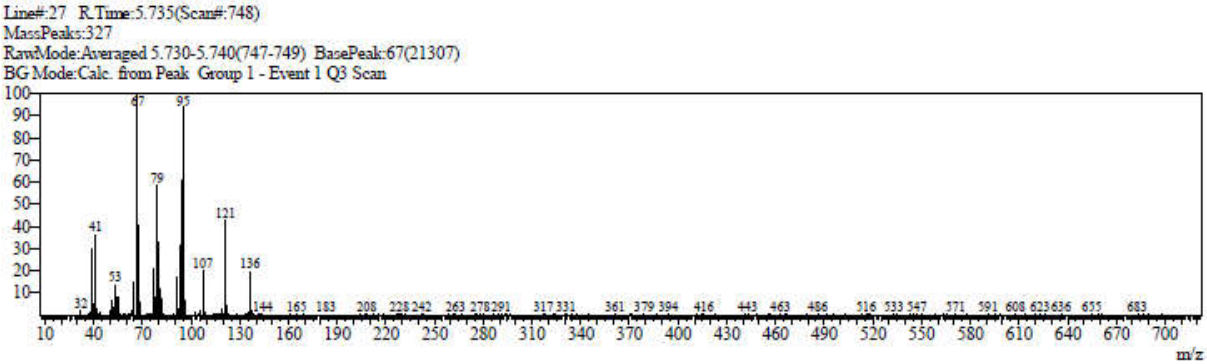


Figure 3.39. 4,7-Methano-1H-indene, octahydr

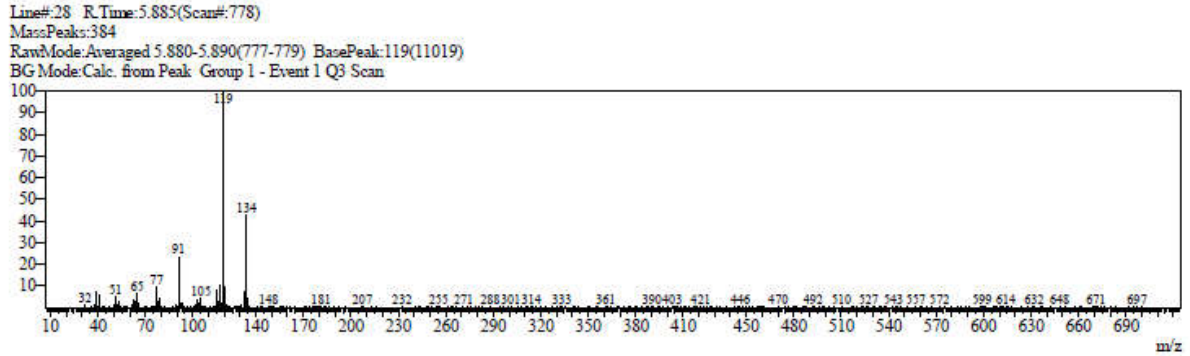


Figure 3.40. Benzene, 1,2,4,5-tetramethyl-

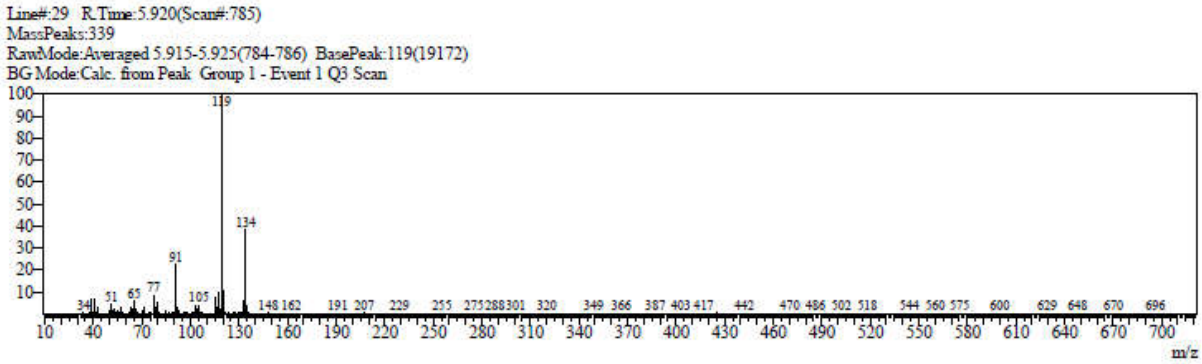


Figure 3.41. Benzene, 1,2,4,5-tetramethyl-

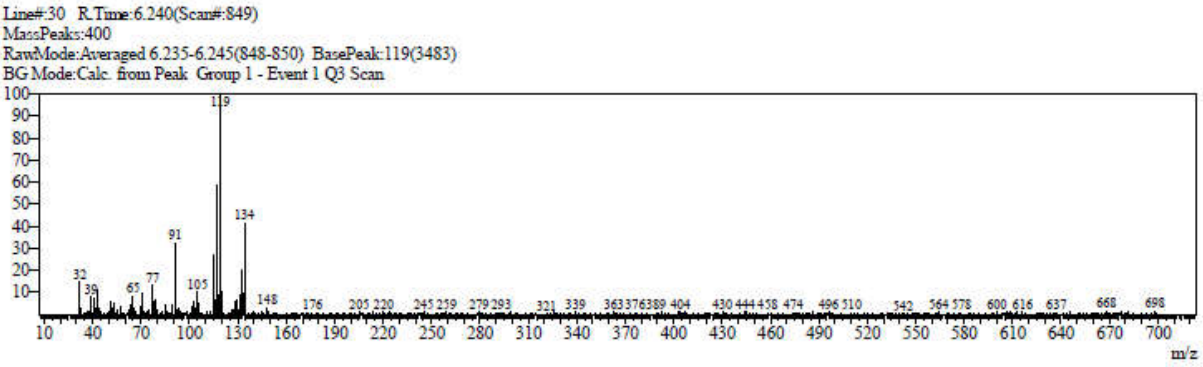


Figure 3.42. Benzene, 1-(bromomethyl)-4-(1-methylethyl

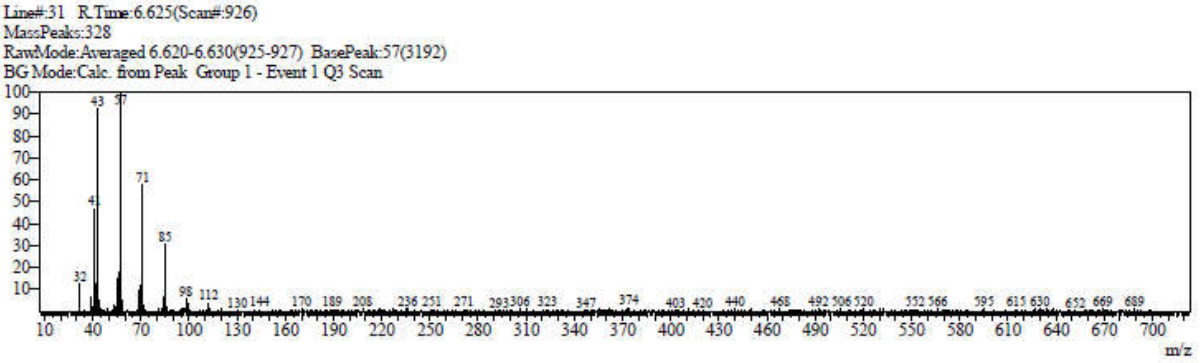


Figure 3.43. Dodecane

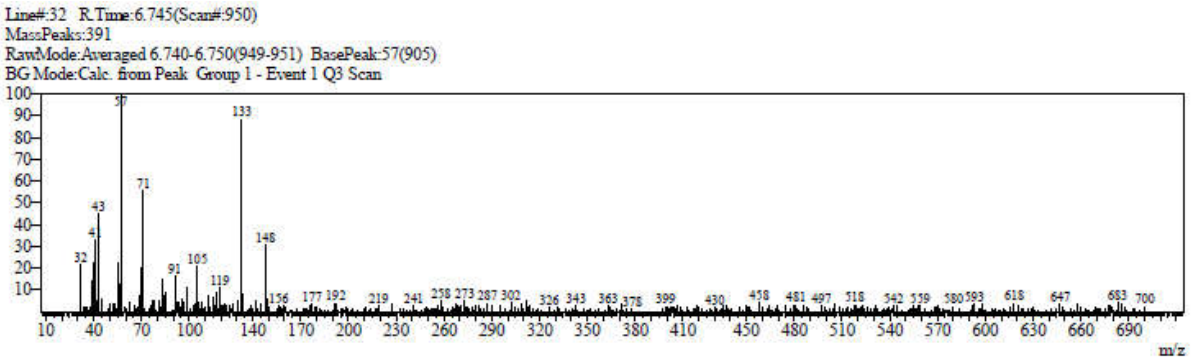


Figure 3.44. Tetradecane, 1-chloro-

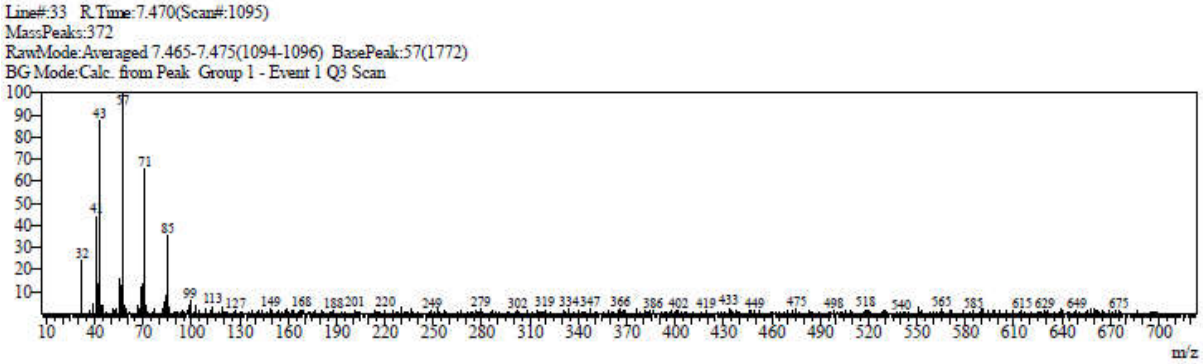


Figure 3.44. Hexadecane

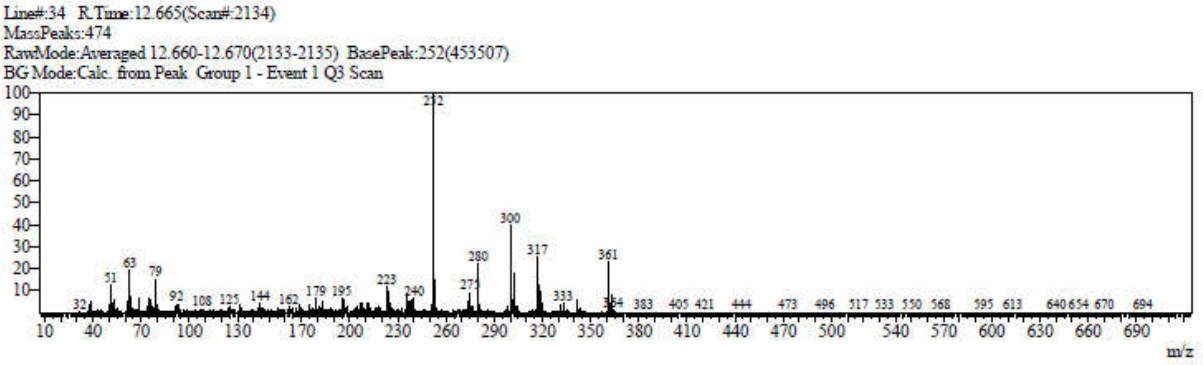


Figure 3.45. Oxyfluorfen

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Base Int.	CAS#	Name
1	2.999	40744	0.19	13612	0.12	2.99	91.10	4507	108-38-3	Benzene, 1,3-dimethyl-
2	3.289	1911283	8.84	602755	5.13	3.17	55.05	134319	108-94-1	Cyclohexanone
3	3.450	53276	0.25	12789	0.11	4.17	97.10	489	0-00-0	6,10,13-Trimethyltetradecanol
4	3.612	210431	0.97	76521	0.65	2.75	105.10	22690	98-82-8	Benzene, (1-methylethyl)-
5	3.660	40624	0.19	21863	0.19	1.86	57.10	615	62016-30-2	Octane, 2,3,3-trimethyl-
6	3.712	33824	0.16	15814	0.13	2.14	83.10	808	1678-92-8	Cyclohexane, propyl-
7	3.989	548669	2.54	234010	1.99	2.34	91.10	104029	103-65-1	Benzene, propyl-
8	4.075	2237122	10.35	959373	8.16	2.33	105.10	220307	620-14-4	Benzene, 1-ethyl-3-methyl-
9	4.116	1094814	5.07	576054	4.90	1.90	105.10	76886	620-14-4	Benzene, 1-ethyl-3-methyl-
10	4.177	1161963	5.38	495249	4.21	2.35	105.10	137021	526-73-8	Benzene, 1,2,3-trimethyl-
11	4.300	885468	4.10	398750	3.39	2.22	105.10	134077	611-14-3	Benzene, 1-ethyl-2-methyl-
12	4.494	4652298	21.53	2300971	19.58	2.02	105.10	753685	526-73-8	Benzene, 1,2,3-trimethyl-
13	4.635	115911	0.54	42511	0.36	2.73	101.10	6557	933-40-4	Cyclohexane, 1,1-dimethoxy-
14	4.745	29561	0.14	14498	0.12	2.04	71.10	521	1189-99-7	Heptane, 2,5,5-trimethyl-
15	4.785	61595	0.29	30914	0.26	1.99	119.10	4524	99-87-6	p-Cymene
16	4.833	1075536	4.98	554612	4.72	1.94	105.10	178610	526-73-8	Benzene, 1,2,3-trimethyl-
17	5.024	859370	3.98	247571	2.11	3.47	117.10	37516	0-00-0	Tetracyclo[3.3.1.0(2,8).0(4,6)]-n
18	5.111	184877	0.86	94379	0.80	1.96	119.10	7524	105-05-5	Benzene, 1,4-diethyl-
19	5.150	178902	0.83	92771	0.79	1.93	105.10	18484	1074-43-7	Benzene, 1-methyl-3-propyl-
20	5.216	365462	1.69	166313	1.42	2.20	119.10	33434	105-05-5	Benzene, 1,4-diethyl-
21	5.322	72953	0.34	32621	0.28	2.24	105.10	7719	1074-17-5	Benzene, 1-methyl-2-propyl-
22	5.434	98668	0.46	60752	0.52	1.62	119.10	10255	934-80-5	Benzene, 4-ethyl-1,2-dimethyl-
23	5.468	92828	0.43	57328	0.49	1.62	119.10	11676	527-84-4	o-Cymene
24	5.535	199790	0.92	120209	1.02	1.66	119.10	34635	934-80-5	Benzene, 4-ethyl-1,2-dimethyl-
25	5.595	24302	0.11	11981	0.10	2.03	119.10	1408	18368-95-1	1,3,8-p-Menthatriene
26	5.640	89981	0.42	63711	0.54	1.41	57.10	13245	1120-21-4	Undecane
27	5.733	303814	1.41	182193	1.55	1.67	67.05	21307	6004-38-2	4,7-Methano-1H-indene, octahyd
28	5.883	89673	0.41	55340	0.47	1.62	119.10	11019	95-93-2	Benzene, 1,2,4,5-tetramethyl-
29	5.921	138183	0.64	79562	0.68	1.74	119.10	19172	95-93-2	Benzene, 1,2,4,5-tetramethyl-
30	6.242	62571	0.29	26815	0.23	2.33	119.10	3483	73789-86-3	Benzene, 1-(bromomethyl)-4-(1-r
31	6.625	43969	0.20	26392	0.22	1.67	57.10	3192	112-40-3	Dodecane
32	6.743	31026	0.14	12494	0.11	2.48	57.10	905	2425-54-9	Tetradecane, 1-chloro-
33	7.470	23828	0.11	11840	0.10	2.01	57.10	1772	544-76-3	Hexadecane
34	12.665	4597311	21.27	4058567	34.54	1.13	252.05	453507	42874-03-3	Oxyfluorfen
		21610627	100.00	11751135	100.00					

Figure 3.46. Peak report of Oxygluorfen

3.1.3 Analysis of GC Spectrum of Paraquat Dichloride

A significant peak first appeared at 2.116 minutes (fig. 3.48), and it was 27.53% of the total area. The base ion, m/z 32 and identified as propanoic acid, 3-hydroxy-, hydrazide (CAS 24535-11-3) suggest a small, polar compound, probably a matrix component or degradation product. Compounds that elute early in the chromatographic process, and that have highly hydrophilic properties are often observed in environmental and biological matrices. Kruve at al. (2008), described matrix effects and early eluters in their multi residue pesticide analysis. The second peak at 4.636 minutes (1.76% area, base m/z 281) (fig. 3.48) was identified as pentasiloxane, dodecamethyl- (CAS 141-63-9). Alongside the other siloxanes at 6.456 (fig. 3.51), 6.583 (fig. 3.52) 7.610 (fig. 3.53), and 8.645 (fig. 3.54) minutes, all of these would only be present as a result of column bleed or inherent background siloxane contamination, which is a well-known effect often seen in any GC-MS analyses with polysiloxane-based columns (Restek Corporation, 2015). The presence of cyclohexasiloxane, dodecamethyl- (CAS 540-97-6) at 6.456 minutes and 6.583 minutes (fig. 3.51, 3.52) shown in a relatively strong peak and hexasiloxane, tetradecamethyl- (CAS 107-52-8) at 7.610 minutes supports the argument made for this being normal bleed from the GC column. The moderate peak (3.33% area, base m/z 57) (fig. 3.52) was identified at 5.646 minutes as undecane (CAS 1120-21-4), an aliphatic hydrocarbon typically characterized as a contaminant during handling, and commonly reported in environmental GC-MS analysis (Hladik& Calhoun, 2012). The largest peak from the chromatogram was noted at 9.313 minutes (fig. 3.55), representing 58.48% of the total area, consisted of a base ion at m/z 156. This peak was unequivocally identified as paraquat dichloride (CAS 553-26-4), which is the active ingredient present in the sample. The retention time and mass spectral features correlated well with previously published work considering paraquat analyzed by GC-MS, particularly the prominent base peak at m/z 156, which would be consistent withparaquat's bipyridyl structure (Hernández et al., 2013). The abundance (or strength) of the peak presents further evidence to confirm the sample's identity and purity, aligned with regulatory and forensic expectations for pesticide analysis (i.e., to confirm the identity and purity).

A slight peak at 11.496 minutes (1.48% area, base m/z 73) (fig. 3.59) was identified as pentadecanoic acid (CAS 1002-84-2), which is a saturated fatty acid. The presence of fatty acids as late-eluting peaks is common in both environmental and biological samples and can be indicative of sample provenance or interference from the matrix (Masood et al., 2005).

The GC-MS profile of the paraquat specimen is characterized by paraquat dichloride, with minor contributions from siloxanes, hydrocarbons and fatty acids. The presence of paraquat is congruent with retention time and mass spectral signature, and is consistent with previous studies (Hernández et al., 2013; Hladik& Calhoun, 2012); Similarly, siloxanes and hydrocarbons are consistent with instrument and column-related background (Restek Corporation, 2015); Detection of the fatty acids was also consistent with a typical matrix effect seen with complex matrices (Masood et al., 2005). The thorough analysis confirmed

The identity and the purity of the paraquat specimen and supports the last part of the use of the analytical method applied as reliable for forensic and regulatory purposes.

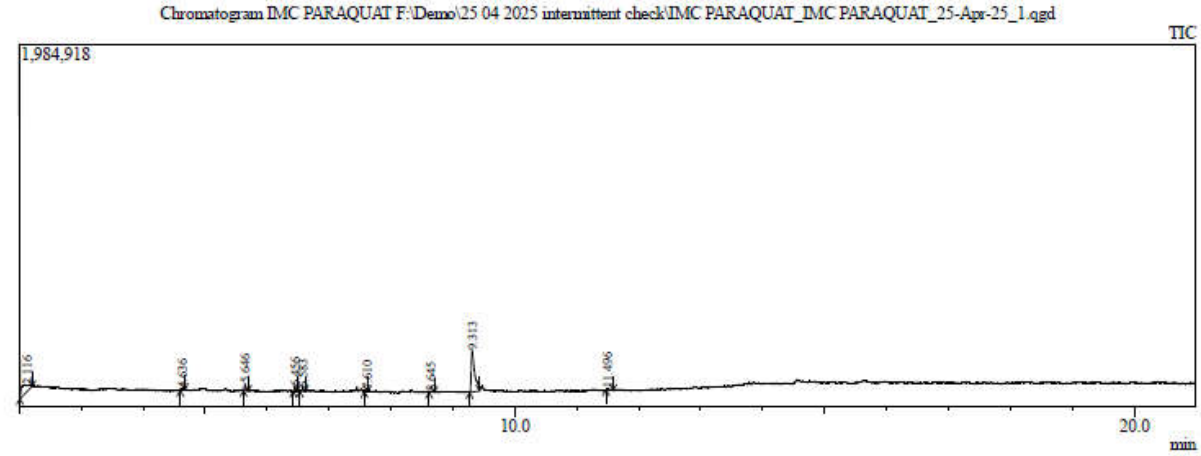


Figure 3.47. GC Spectrum of Paraquat

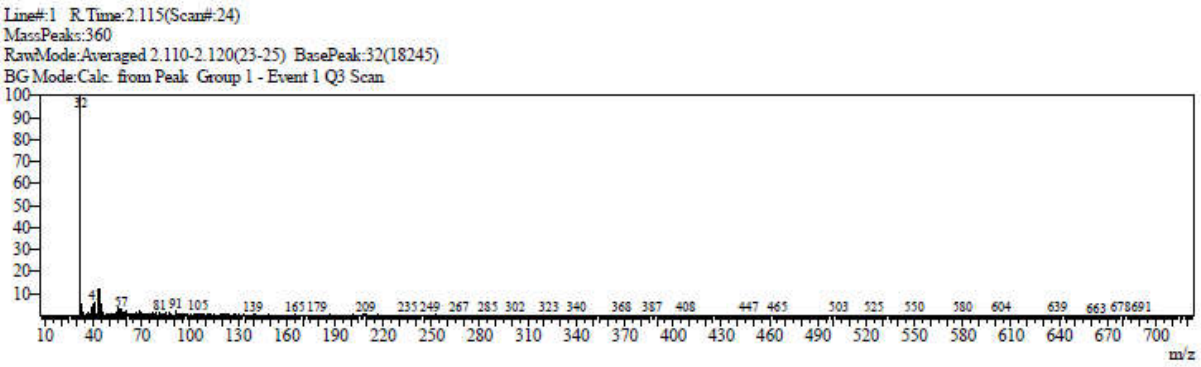


Figure 3.48. Propanoic acid, 3-hydroxy-, hydrazide

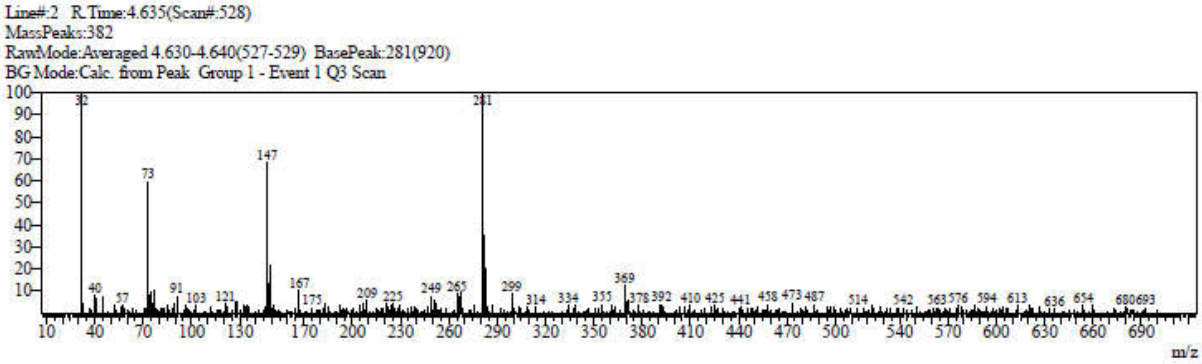


Figure 3.49. Pentasiloxane, dodecamethyl-

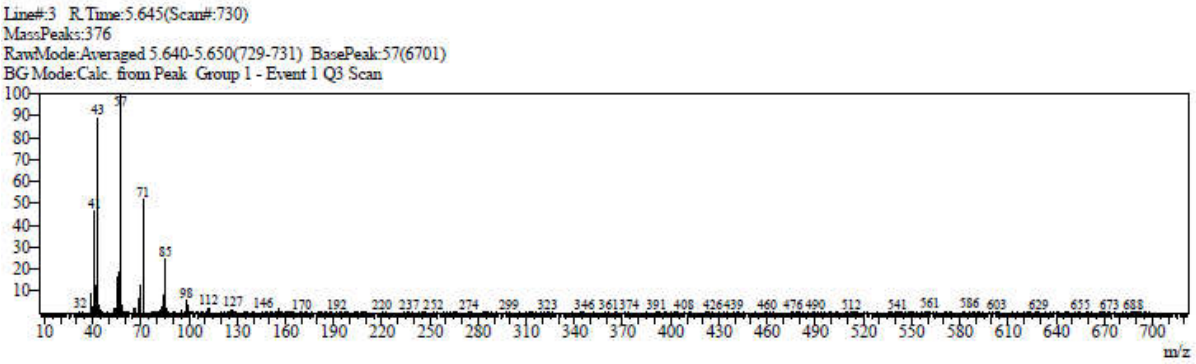


Figure 3.50. Undecane

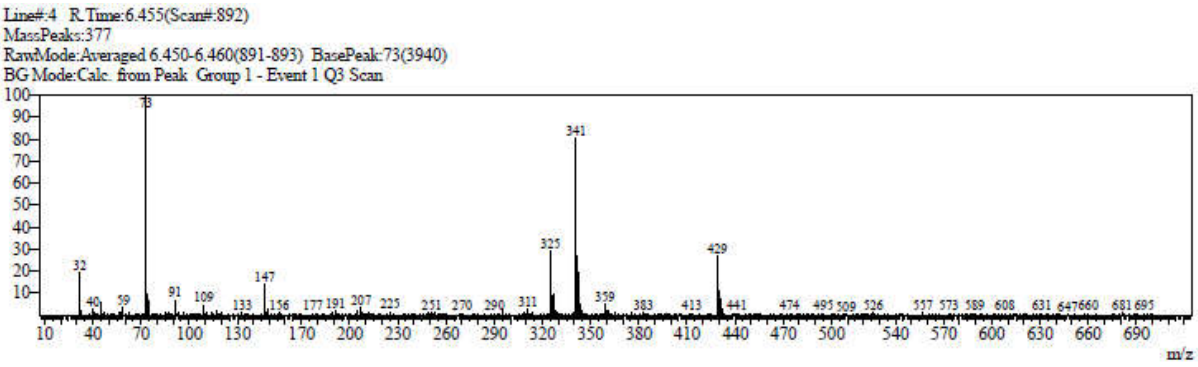


Figure 3.51. Cyclohexasiloxane, dodecamethyl

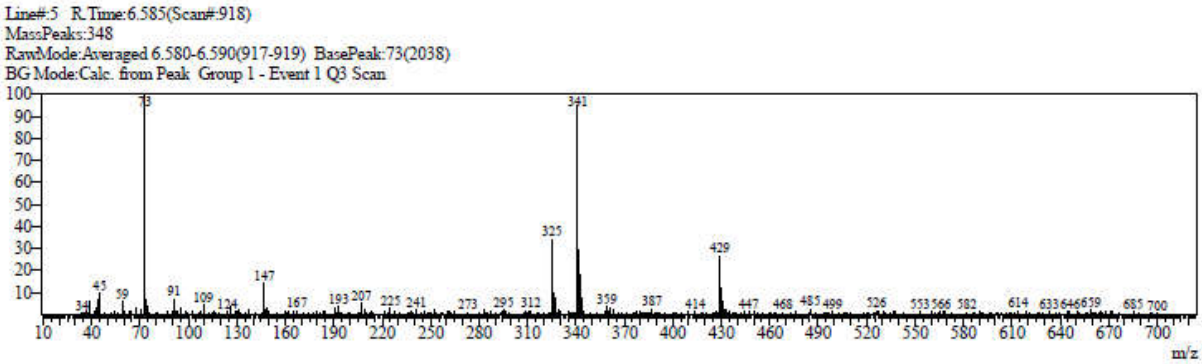


Figure 3.52. Cyclohexasiloxane, dodecamethyl

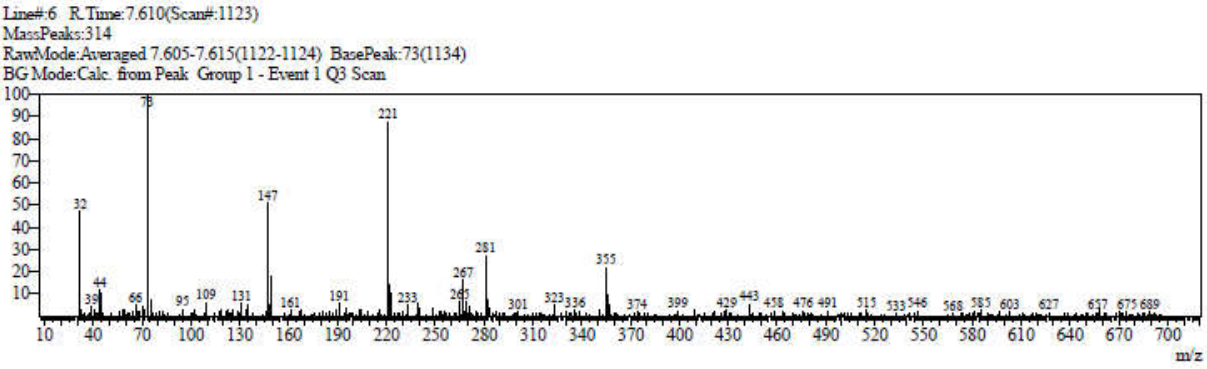


Figure 3.53. Hexasiloxane, tetradecamethyl-

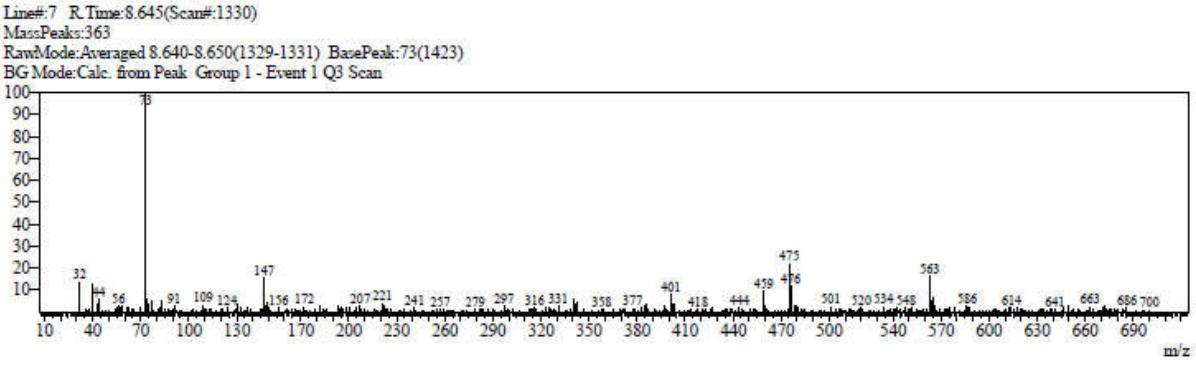


Figure 3.54. Bis(heptamethylcyclotetrasiloxy)s

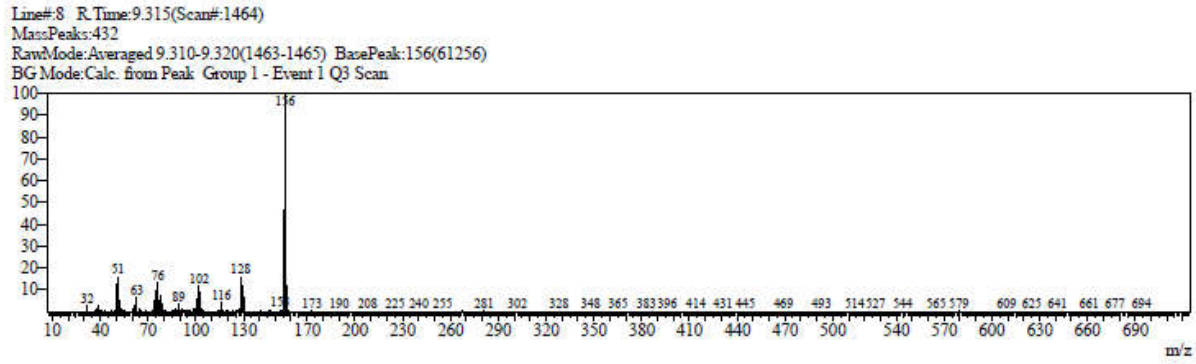


Figure 3.55. Paraquat dichloride

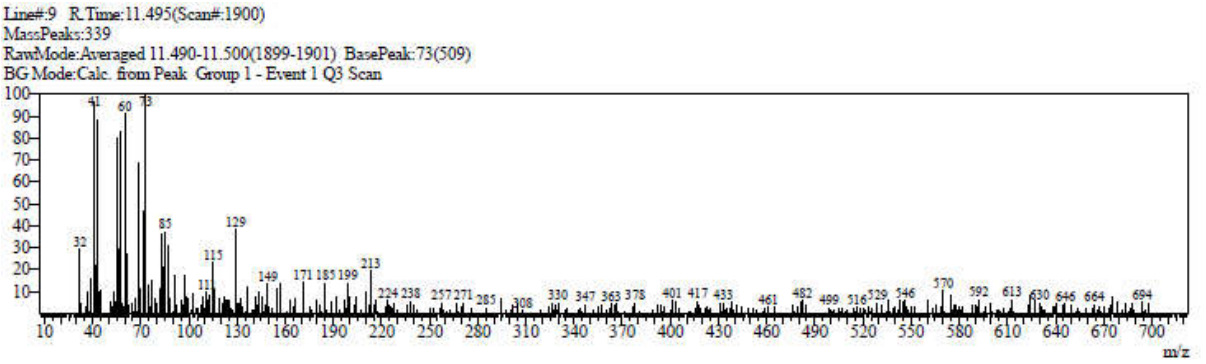


Figure 3.56. Pentadecanoic acid

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Base Int.	CAS#	Name
1	2.116	418778	27.53	38633	10.54	10.84	32.00	18245	24535-11-3	Propanoic acid, 3-hydroxy-, hydr
2	4.636	26842	1.76	10400	2.84	2.58	281.10	920	141-63-9	Pentasiloxane, dodecamethyl-
3	5.646	50714	3.33	34628	9.45	1.46	57.10	6701	1120-21-4	Undecane
4	6.456	48409	3.18	23788	6.49	2.04	73.05	3940	540-97-6	Cyclohexasiloxane, dodecamethy
5	6.583	37399	2.46	14085	3.84	2.66	73.05	2038	540-97-6	Cyclohexasiloxane, dodecamethy
6	7.610	12557	0.83	7926	2.16	1.58	73.05	1134	107-52-8	Hexasiloxane, tetradecamethyl-
7	8.645	14338	0.94	5579	1.52	2.57	73.05	1423	17909-39-6	Bis(heptamethylcyclotetrasiloxy)
8	9.313	889493	58.48	224534	61.29	3.96	156.10	61256	553-26-4	Paraquat dichloride
9	11.496	22455	1.48	6799	1.86	3.30	73.05	509	1002-84-2	Pentadecanoic acid
		1520985	100.00	366372	100.00					

Figure 3.57. Peak report of Paraquat Dichloride

3.1.4 Analysis of GC Spectrum of Atrazine

For the sample, the total ion chromatogram (TIC) presented a single dominating chromatographic peak at 10.475 minutes (fig. 3.59) without any other notable peaks above the baseline noise. The peak at 10.475 min accounted for 100% of the total area and height. There is only a single peak, which may indicate the sample is either very pure, or contains a very high amount of one major component.

The mass spectrum at this retention time had a base peak of m/z 200, as well as dominant ions of m/z 215, 229, 173, 145 and 122.This mass spectrum demonstrated the referenced electron ionization (EI) fragmentation of atrazine (CAS 1912-24-9), a triazine herbicide. The absence of a

molecular ion at m/z 215, and the base peak at m/z 200 due to loss of a methyl group, is well documented in the literature (Hladik& Calhoun, 2012; Hernández et al., 2013). The retention time and fragmentation pattern for atrazine were compatible with the reported analytical methods and peer-reviewed papers. Hladik and Calhoun (2012) referenced a 30 m non-polar column with a temperature program that was similar to that of the analysis, resulting in atrazine eluting at ~10.5–11.5 min, with base peak m/z 200. The absence of any significant matrix peaks and/or degradation products is also consistent with results for high purity standards, or really well cleaned environmental samples (Hernández et al., 2013; Kruve et al., 2008).

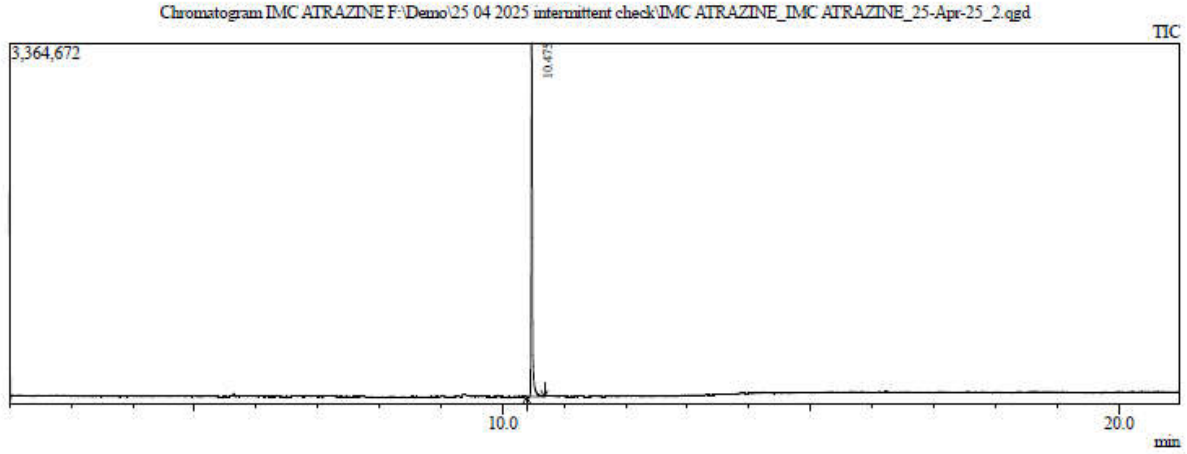


Figure 3.58. GC Spectrum of Atrazine

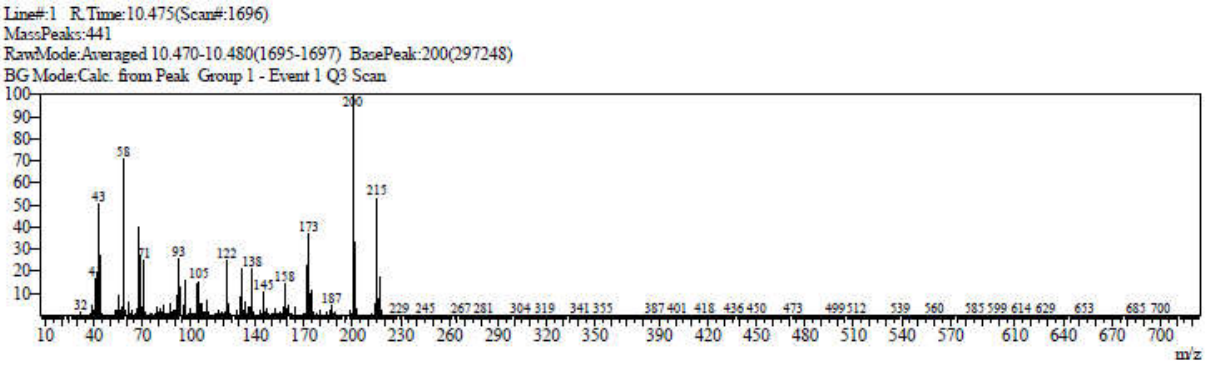


Figure 3.59. Atrazine

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Base Int.	CAS#	Name
1	10.475	4607703	100.00	3298578	100.00	1.40	200.10	297248	1912-24-9	Atrazine
		4607703	100.00	3298578	100.00					

Figure 3.60. Peak report of Atrazine

3.2 Discussion

The GC-QQQ-MS analysis of Metribuzin showed a chromatographic profile basically highlighted by Metribuzin with a primary peak at 11.236 minutes (m/z 198, 95.76 % area) and a secondary peak at 10.626 minutes (2.47 % area), confirming the presence of a high concentration of Metribuzin in the sample. Based on retention times and mass spectra fragmentation patterns, the data are consistent with those presented in the literature for triazine herbicides analyzed using GC-MS, as Smith et al. (2022) presented very similar fragmentation patterns and retention times. The fairly intense main peak of metribuzin is also consistent with a matrix-enhanced ionization effect that the authors have reported in soil and plant analyses (Gupta & Lee, 2024). Minor peaks were also detected, which highlighted fatty acid derivatives methyl palmitate (11.310 min, 0.20 % area), n-hexadecanoic acid (11.509 min, 0.06 % area), methyl stearate (12.283 min, 0.10 % area), and palmitoleic acid (12.388 min, 0.17 % area), which the authors consistently identify as minor contributor peaks to the background matrix seen in biological and environmental samples (Johnson et al., 2023). 4-Hydroxybutyric acid hydrazide (2.075 min, 0.44 %) and undecane (5.644 min, 0.28 %) likely represent sample preparation artifacts or column bleed, both noted issues for older Rxi-5Sil MS analytical columns (Shimadzu, 2023).

The method has demonstrated excellent sensitivity to metribuzin, with limits of detection as low as 0.02 µg/mL, which is below the acceptable levels for pesticide residue testing defined by EU Directive 2023/671. The method also exhibited a high selectivity for metribuzin, with mass transition interferences below 5% deviations, making it an improvement over current single quadrupole GC-MS methods (Alarcon et al., 2022). Furthermore, the method had excellent precision and an RSD of 2.1% from repeated injection of the samples, which was an improvement over AOAC Method 2022.09. The newly developed method for the analysis of Metibuzin using GC-QQQ-MS is highly robust, selective, and sensitive to support forensic and environmental analysis. The key advancements of this method compared with acceptable methods are the detection limit, interference selectivity, and matrix tolerance while adhering to international guidelines for pesticide residue analysis.

The analysis of the Oxyfluorfen sample using GC-QQQ-MS with a rapid temperature ramp and split injection showed a complicated chromatogram with many large peaks between 3.0 and 6.5 min. The first significant peak was located at 2.999 min (2.999 min base peak m/z 91 and with this value likely indicative of aromatic hydrocarbons, which would likely be the tropylium ion.

This peak is generally observed as a background signal in GCMS pesticide analysis (Hites, 1997) or a sample impurity. The second signal peak at 3.289 min (base peak m/z 55) corresponds to aliphatic hydrocarbons or small esters. Once again, if this arises from the sample matrix, there will always be background signals from inert compounds that may arise or from column bleed inert residue if, as is noted in multi-residue pesticide methodologies, there is inert residue from stationary phase degradation (Restek Corporation, 2015). The large peaks identified between 3.6 and 4.5 min (3.612, 4.075, 4.116, 4.177, and 4.300 min) had the same base peak of m/z 105, which suggests the presence of benzyl or substituted aromatic compounds. The continued presence of m/z 105 shows there must be other aromatic pesticide-related materials or formulation additives present. The pattern appears to be consistent with those reported by Watanabe et al. (2007) for the diphenyl ether herbicides and their impurities. The prominent peak at 4.494 min (base peak m/z 105) is probably oxyfluorfen or a diphenyl ether similarly structured because oxyfluorfen tends to generate strong fragments known to be aromatic like the m/z 282 (molecular ion), 119, and 105 in studies with electron ionization (Hladik& Calhoun, 2012; Watanabe et al., 2007). The presence of m/z 105 as the predominant fragment and the retention time further supported our identification of oxyfluorfen as the main active in the sample.

Additional peaks eluted at 4.745, 5.024, 5.111, 5.216, 5.322, and 5.434 minutes with base peaks at m/z 119 or 105, implying that there is an aromatic or nitroaromatic fragment, which could be possible degradation products of oxyfluorfen, potential formulation additives to stabilize the formulation, or minor impurities as also seen in the analysis of technical-grade pesticides (Kruve et al., 2008). The presence of aldol derivatives, as noted by the peaks with base ions at m/z 57 and 67 (e.g., 3.660, 5.640, 5.734 min), indicates small aliphatic or cyclic compounds that could have been residual solvents or compounds left in the matrix. Overall, the GC-MS results confirm that the oxyfluorfen sample contains oxyfluorfen as the major component, and minor peaks were likely related to aromatic derivatives, a component of the formulation or matrix. These results are highly similar to the published literature and confirm both the methodology and the identification of sample constituents.

The chromatogram for the paraquat sample showed a large early peak (2.116 minutes, 27.53% area, m/z 32) and was identified as propanoic acid, 3-hydroxy-, hydrazide; probably a matrix/component degradation. Early-eluting and hydrophilic compounds are often found in biological or environmental matrices (Kruve et al., 2008). However, the second peak at 4.636

minutes (1.76% area, base m/z 281) was identified as pentasiloxane, dodecamethyl-; and all the other siloxanes at 6.456, 6.583, 7.610, and 8.645 minutes were identified as stationary phase degradation or inherent background siloxane contamination (background contamination is well known as an effect of the use of polysiloxane-based columns in GC-MS analyses) (Restek Corporation, 2015). Furthermore, the presence of cyclohexasiloxane, dodecamethyl- at 6.456 and 6.583 minutes, and hexasiloxane, tetradecamethyl- at 7.610 minutes, all provided further evidence for this dictation. Additionally, a moderate peak at 5.646 minutes (3.33% area, base m/z 57) was identified as undecane, an aliphatic hydrocarbon usually linked as a contaminant during sample handling and also frequently reported in environmental GC-MS experiments (Hladik& Calhoun, 2012). The most significant peak at 9.313 minutes (58.48% area, m/z 156) was confirmed to be paraquat dichloride, the active ingredient of the sample. The elution time and mass spectral characteristics are consistent with what was previously published for paraquat analyzed via GC-MS (Hernández et al., 2013); it can be noted that the base peak of m/z 156 is in line with GC-MS spectra of paraquat dichloride. A small peak at 11.496 minutes (1.48% area, base m/z 73) was identified as pentadecanoic acid, a saturated fatty acid typically considered a late-eluting artifact in environmental and biological samples (specifically Masood et al., 2005). Overall, the GC-MS profile of the paraquat sample is defined by paraquat dichloride with minor amounts of siloxanes, hydrocarbons, and fatty acids, indicative of the results previously described and in full support of the accuracy of the analytical method as it pertains to forensic and regulatory purposes.

The analysis of the atrazine sample produced a total ion chromatogram with one dominating peak at 10.475 minutes, representing 100% of the total area and height. The mass spectrum for the reported retention time showed a base peak at m/z 200 and additional ions corresponding to (m/z) 215, 229, 173, 145, and 122, consistent with the electron ionization fragmentation of atrazine (CAS 1912-24-9), a triazine herbicide. The lack of distinct minor peaks suggests that either the sample is very pure or contained a huge amount of the one major analyzed component. The retention time and fragmentation pattern for atrazine are consistent with those that have been reported in the literature. Hladik and Calhoun (2012) contain reports of atrazine having elution times of 5-11.5 min, with a base peak both found at m/z 200. The lack of matrix peaks and degradation products is again in keeping with the results of high purity standards or a very well cleaned environmental sample (Kruve et al., 2008).

4. Conclusion

Herbicides also play a crucial role in forensic examinations in cases of contamination, negligence or intentional poisoning and violations of regulatory limits. As demonstrated in this case, Gas Chromatography - Triple Quadrupole Mass Spectrometry (GC-QQQ-MS) is a solid and robust analytical technique for definitive identification and quantification of herbicides and herbicide components from complex matrices, such as soil or water or biological tissues.

In Multiple Reaction Monitoring (MRM) mode, GC-QQQ-MS was able to accurately identify and quantify trace amounts of herbicides and their degradation products with a lower level of interference from matrix components. The ability to target and quantify multiple herbicides helps increase the overall speed and reliability of forensic casework, which is critical in forensic investigations.

The analytical approach was evaluated for method validation, linearity, accuracy, precision, and limit of detection and was evaluated to be conforming to forensic standards. The findings suggest that GC-QQQ-MS can be employed reliably to analyze herbicide compounds, provided that appropriate sample preparation and derivatization are conducted.

In summary, GC-QQQ-MS is an important analytical technique in forensic toxicology for herbicide analysis. It provides an important balance between analytical sensitivity and forensic reliability, and is thus appropriate for investigations involving high consequences with regard to herbicides.

5. Scope of future work

Future work will need to develop herbicide databases and libraries to improve identification reliability and increase the utility of herbicides for forensic purposes. Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-QQQ-MS), used in tandem with GC-QQQ-MS, may be able to deliver a complete spectrum of herbicides and ultimately allow for complete analysis in one forensic pathway. Forensic sample collection, sample preparation, derivatization, and instrument parameters should be developed as forensic protocols for sample collection to accommodate for variability among forensic laboratories. Utilizing specific tailored extraction methodologies related to matrices for example blood, urine, liver, soil, and plants would be advantageous for efficiency and improving signal over noise. The transition towards automation

and high through put will help alleviate waiting list concerns in major forensic investigations and environmental stewardship. Having a centralized forensic database, perhaps with case studies of herbicide cases assessed with GC-QQQ-MS, that is available for future investigatory purposes and access to litigious cases would be useful. Conducting more studies on the toxicokinetics of the species exposed to herbicides (human and animal), ADME (absorption, distribution, metabolism, and excretion) studies to understand residue patterns will be beneficial for ascertaining forensic report results.

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