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How to read and Interpret GC/MS Spectra

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ABSTRACTS

Gas chromatography mass spectrometry (GC/MS) analysis is used as analytical tool for volatile and complex mixture samples. Its effectivity and sensitivity to detect the analyte components make it applicable in a wide range of purposes. This technique can be used to conduct quantitative as well as qualitative analysis. Unfortunately, very few, if there are, of publications dedicate the research to give an explanatory report using a step-by-step approach on how to read and interpret the analysis result. Thus, this study is aimed to fulfill the gap on bridging the spectra data output of GC/MS measurement into the light of easy way reading and interpreting its spectra. The research method used both library spectra matching and a priori spectra interpretation on chosen compound samples. The general guideline and application are discussed.

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1. INTRODUCTION

Gas chromatography mass spectrometry (GC/MS) analysis is an effective testing and troubleshooting tool to identify and quantify chemicals in a complex mixture (Al-Rubaye *et al.*, 2017). Some of technical fields using GC-MS analysis are perfume industry (Van Asten, 2002), food (Chiu & Kuo, 2020; Vene *et al.*, 2013), hydrocarbon fuels research pharmacy research (He *et al.*, 2016), forensic (Shimadzu, 2011; Bridge *et al.*, 2018), and sometimes it becomes more powerful to detect chemical warfare agent (Li *et al.*, 2020).

GC and MS provide distinct but complementary results; while GC separates components of a mixture, MS can analyze and identify these components. These methods were first used in tandem in the 1950s, and are still widely applied in clinics and laboratories worldwide (Simon-Manso *et al.*, 2013).

Additionally, the separation by gas chromatography also provides information and contributes to an unambiguous identification of organic substances. The process of identification is nowadays supported by automated search algorithms; however, a profound identification needs an in-depth knowledge of interpretation of both mass spectrometric and gas chromatographic data. Therefore, this study is addressed to collect and summarize the way to read and interpret GC/MS output in a practical and systematically manner. The principal aspects of GC/MS data interpretation are given in advance by a brief description of up-to-date processes of identification and, finally, by some examples from laboratory research background and industrial uses as technical matter analyses.

1.1. Current knowledge for understanding GC/MS spectra

GC/MS is the synergetic combination of two powerful microanalytic techniques for complex mixture (Bridge *et al.*, 2019). The gas chromatograph separates the components of a mixtures in time, and the spectrometer provides useful information on structural identification of each component and quantifies the chemicals (Williams *et al.*, 2014). Several advantages resulting from this combination. Qualitative purposes can be obtained by complex mixture separation with individual compound mass spectra showing a characteristic fragmentation pattern or “chemical” fingerprint; quantitative information of the compound collected in the same time from its nominal masses (Wu *et al.*, 2013).

There are two types of data output (spectra) from different processes in the GC/MS analysis:

- a) *Chromatogram*. It describes a sequential peaks of separated analyte components at different time (called as Relative Retention Indexes, RRI) resulted from different level of attraction between the compounds and the column as shown in **Figure 1**. Every single one of the chromatogram peaks represents a relative pure element of the sample after separated chromatographically.
- b) *Fragmentogram* (or mass spectra). The spectra show the pattern of mass fragmentation of the detected elutants molecule ions as described in **Figure 2**. Fragmentation is the most important process that allows to obtain information on the molecular structure of the analyte measured by mass spectrometry (Holcapek & Byrdwell, 2017).

In order to achieve a successful structural determination, the mass spectrum should be of a pure analyte (Sinha *et al.*, 2011b). Total of ion current of the detected components become the basic for measuring its relative concentration in the mixture as the distillation model analysis gives a coherent and reliable measurement for the detected elutant as illustrated in **Figure 3** (Smith *et al.*, 1977).

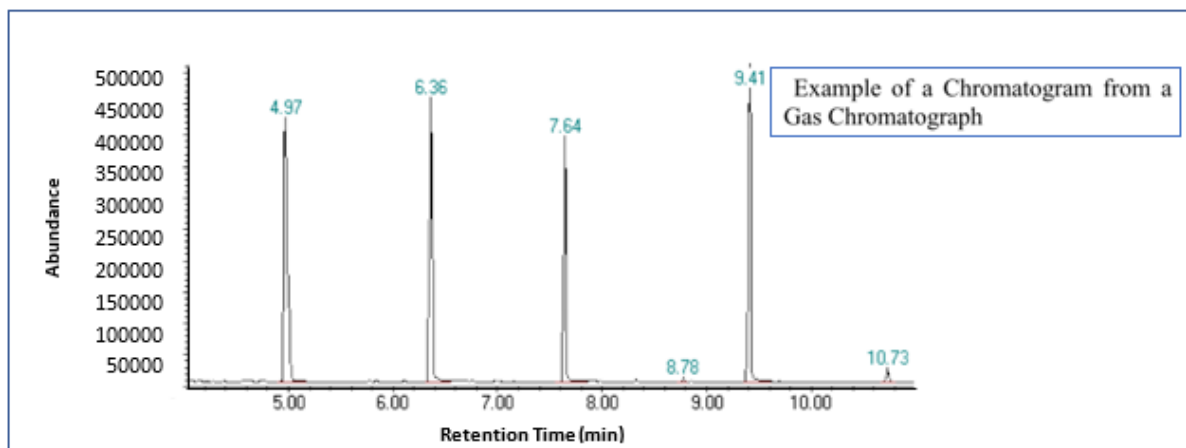


Figure 1. Example of a GC/MS chromatogram displaying compound peaks sequence that separated chromatographically.

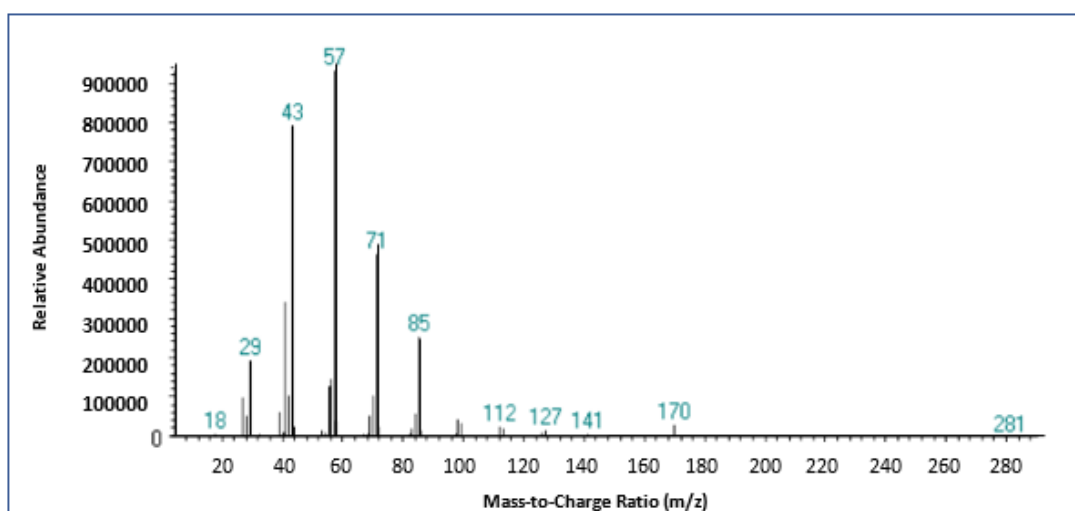


Figure 1. Example of mass spectrum from a mass spectrometer.

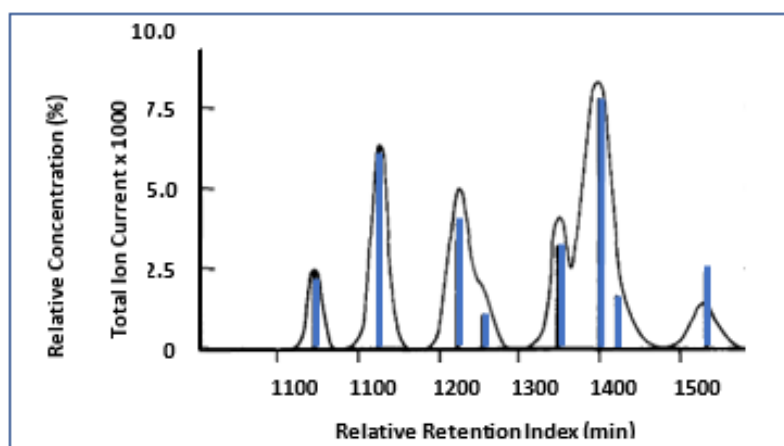


Figure 3. A schematic representation of a GC/MS profile, displaying relative concentration of detected elementary compounds against its relative retention index, superimposed on the corresponding total ion current plot (Smith *et al.*, 1977).

In a GC/MS qualitative analysis, there should be at least two spectra on either side of the spectrum. Consistency of the spectrum to spectrum and the peak shape determine the possibility of compound constitution, either the chromatogram represents a single compound or more than it. Noise spikes, sometimes, are resulted by a neutral particle that is not removed by the vacuum system striking the detector at the time ion of a specific m/z value should be reaching it. It is distinguishable due to none of isotope peak associated. The intensity of the noise peak can have a broad range from several orders of magnitude greater than of the base peak of the analyte to as small as less than 1% of the density of the base peak.

The prerequisite and key challenge of highly complex sample profiling is the rapid, reliable and unambiguous identification (Schauer *et al.*, 2005). The spectral retention index (RI) for the unknown substances can be determined and matched with the database containing values of compounds as the reference, for instance, the National Institutes of Standards and Technology (NIST) which is updated every 3 years. This RI database is a useful tool to identify the analyte along with the values based on literature citations and the GC/MS method used in the procedure to obtain the values.

2. METHODS

2.1. Step-by-Step Interpretation Procedure of Analyte Chromatogram

GC/MS analysis has some optional means of sample introduction, including static headspace analysis, thermal desorption, and direct injection (Al-Rubaye *et al.*, 2017). They can be opted based on type of sample and desired outcome. The resulting chromatograms may differ in term of their appearance, but the basic understanding remain the same. The systems and parameters used for an analysis need to be similar when comparing the results. For instance, the comparison of failed epoxy and its reference spectra in a manufacturing company (Figure 4) resulted from similar parameters (Pellett *et al.*, 2018).

For explorational analysis, the procedure to interpret the GC chromatogram are as follows:

- i. Step 1: Identify the peaks display. There should be at least two spectra to make a certain judgement that analyte mixture has been separated well by the column. Every single one of peak represents separated compound from the mixture.
- ii. Step 2: Identify the order of the peaks along the RI axis. The order of the peaks is based on their boiling points, except for isomers and molecules with similar molecular mass which can be influenced by the method or column used on the compound retaining time. The lowest one will be appeared as the first peak in the left chromatogram since they have the lowest retention time. For commonly known compounds, we can compare the analyte spectra pattern with internal references or any comparable laboratories data.

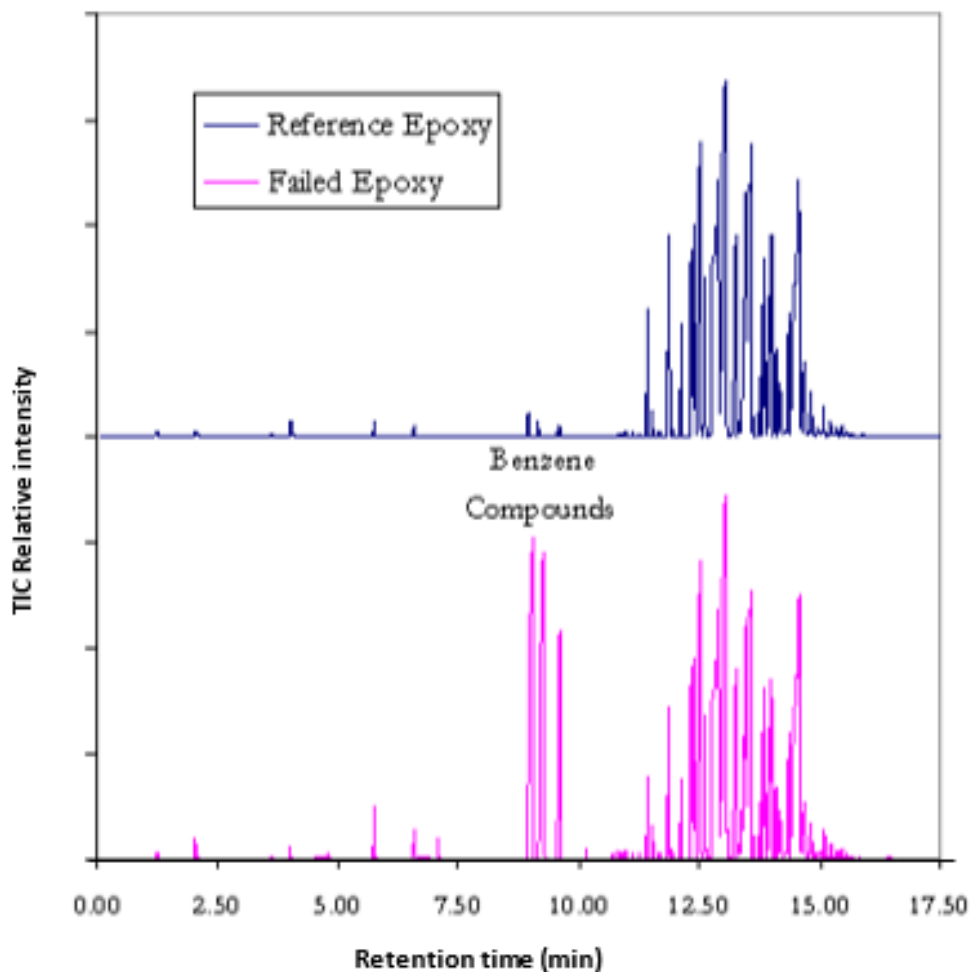


Figure 2. An illustration of different spectra: a reference epoxy (top) and the analyzed epoxy (bottom). Failed epoxy shows its large impurity of benzene compounds (y-axis) detected around 9-minute mark of retention timeline (x-axis).

2.2. Step-by-Step interpretation procedure of mass spectra

After analyze the probability of detected compound through GC chromatogram, the next step is to determine the structure based on mass spectra interpretation. Always read the mass spectrum from the right to the left. The peaks at the highest m/z values in the spectrum represent ions that contain the most information about the intact analyte. Following are steps used in the m/z value determination for a molecular ion peak:

- a) **Step 1:** Identify the molecular ion. The molecular ion ($M^{+\bullet}$) peak, if present, will be the peak at the highest m/z value in the spectra (**Figure 5**). That does not represent sample background or that is not an isotope peak. In an EI mass spectrum, the $M^{+\bullet}$ peak represents an odd-electron ion because all neutral molecules have an even number of electrons, and the $M^{+\bullet}$ is formed by the loss of a single electron from the neutral molecule to form a particle that has a net-positive charge. The exception is for the compounds containing an odd number of N atoms in addition to C, H, O, Si, S, P, and halogens will have an odd nominal mass; therefore, the $M^{+\bullet}$ peak for these compounds will have an odd m/z value as shown in **Figure 6**. Any compound containing an even number of nitrogen atoms will have an even m/z value $M^{+\bullet}$ peak.

- b) **Step 2:** Identify the fragmentation pattern. $M^{+\bullet}$ peak must be followed (right to left) by non-isotope peaks that represent losses of logical groups of atoms based on nominal mass and valence rules. The compounds are fragmented in a certain sequence. An aliphatic chain always shows significant series of ion. For example, **Figure 5** represents 1,1-dichloroethane has fragments pattern as 27, 63, 83, 98. Its mass losses are 69-35-15. Thus, the probability causes of its losses are Cl_2 , Cl, and CH_3 (indicative as C1 aliphatic alkyl fragment).
- c) **Step3:** Determine for significant ion series. Aliphatic series has significant ion series which is easy to identify. The indicative ion series of molecular fragmentation seen in **Table 1**. **Figure 6** shows the fragment peak as 114 m/z value. Its losses due to aliphatic chain by 71 m/z (resulted from 185-114). The number indicative for alkanes significant series.
- d) **Step 4:** Identify the isotope pattern (S, Cl, Br). Chlorine and bromine are very important elements in organic many organic analyses, especially for pollutants detection. Both relative stable isotopes ($^{35}Cl/^{37}Cl$ by ratio 3:1; and $^{79}Br/^{81}Br$ by ratio 1:1, respectively) exhibit significant A+2 pattern with huge abundances. In **Figure 5**, observed from the isotope peak that in molecular ion has typical pattern of exist Cl_2 . **Figure 7** shows a comparison of mass spectra pattern of Cl-isotopes.
- e) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. **Figure 6** shows that nitrogen as the hetero atom can induce the cleavage of nearest bond. The fragment is detected in the spectra.

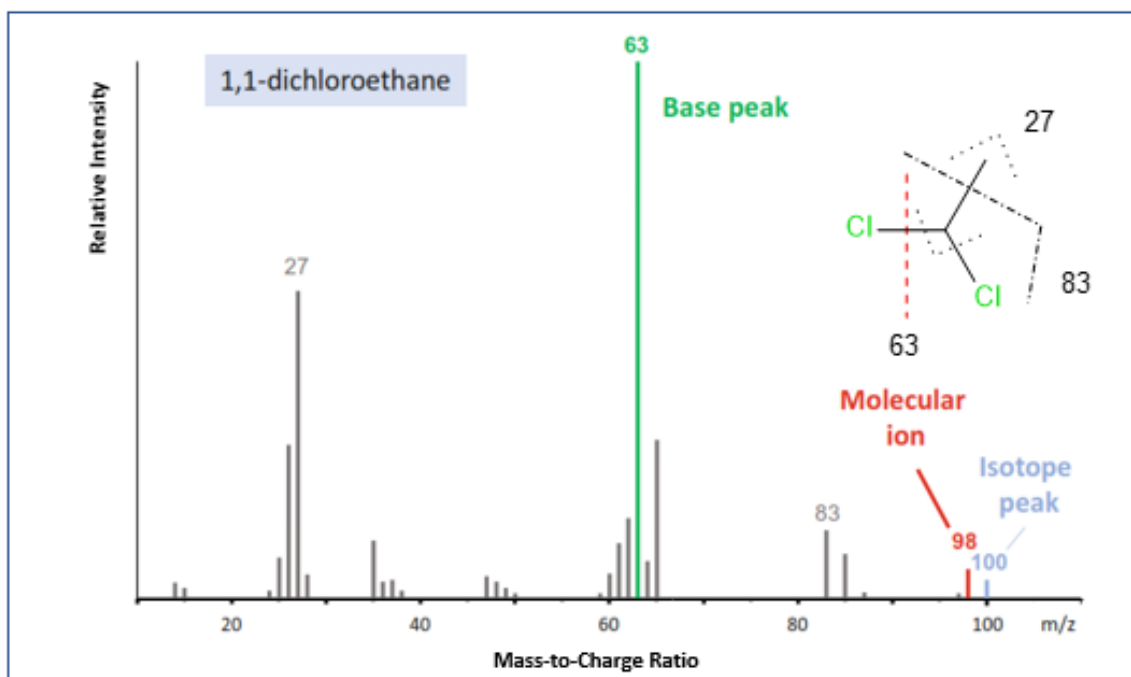


Figure 3. Scheme of typical mass spectrum adopted from Schwarzbauer (2020) with modification.

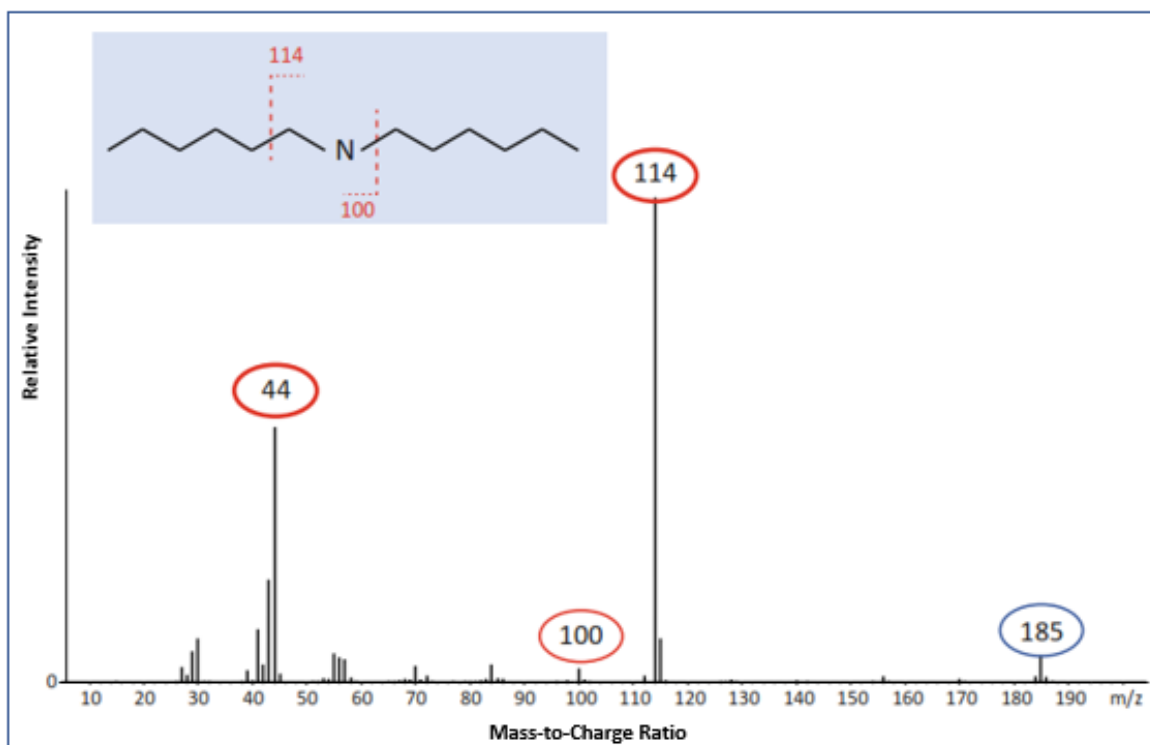


Figure 4. Mass spectra illustration of dihexylamine compounds following N rule (Scwarzbauer, 2020).

Table 1. Indicative fragment patterns on mass spectra identification.

Functional group	Start mass	Ion series
Alkanes	29 (C ₂ H ₅ ⁺)	43, 57, 71, 85, 99, 113, ...
Alkenes	27 (C ₂ H ₃ ⁺)	41, 55, 69, 83, 97, 111, ...
Ethers	31 (CH ₃ -O ⁺)	45, 59, 73, 87, 101, ...
Alcohols	31 (H ₂ C ⁺ -OH)	45, 59, 73, 87, 101, ...
Ketones	43 (CH ₃ -C ⁺ O)	43, 57, 71, 85, 99, 113, ...
Amines	30 (H ₂ C=N ⁺ H ₂)	30, 44, 58, 72, 86, 100, ...
Benzene	77 (C ₆ H ₅ ⁺)	77

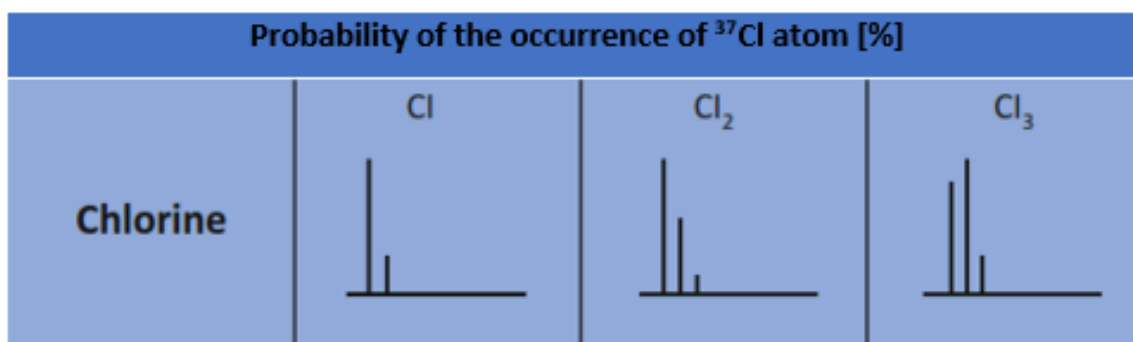


Figure 5. Isotopes pattern of chlorine.

2.3. Experimental Methods

To understand how to read and interpret the GC/MS spectra, the step-by-step procedures are applied to some studies conducted through GC/MS analysis. A hierarchical complexity approach presented to achieve better comprehension as the case study started from the least to the most complex mixtures and compounds structure in the analysis. The simple compounds used to identify are: carbon disulfides, ethanol, acetaldehyde, and dichloro(silyloxy) silane. The fairly complex compounds used are: methyl-tert-butyl-ether (MTBE), methyl-oleic-ester, methyl-palmitic-ester and 1-(2-Hydroxyethyl)-2-imidazolidinone (HEIA). The more complex compounds used are: β -cymene, 1,2,3-benzenetriol, and eugenol. The very complex compounds used are: Diazepam, nordazepam, oxazepam, thiabendazole, and testosterone.

CS₂ spectra used here taken from [Mujawar et al., \(2014\)](#) in a chili mixture containing pesticide compounds. Ethanol and acetaldehyde spectra resulted from a mixture of neural tissue in mouse brain ([Heit et al., 2017](#)). Dichlorosilane spectra identification used from a mixture of trichlorosilane with impurities contain ([Cambria & McManus, 1990](#)). MTBE is identified from gasoline mixture ([Quach et al., 1998](#)). Methyl-oleic ester and methyl-palmitic ester spectra are used from a mixture of fatty oil in tomato seed ([Botinestean et al., 2012](#)). 1-(2-Hydroxyethyl)-2-imidazolidinone (HEIA) spectrum is obtained from post-combustion products mixtures identification ([Saeed et al., 2017](#)). β -cymene compound spectrum is used from the identification of the volatile organic compounds (VOCs) from *Glycyrrhiza uralensis* and honey-roasting products ([He et al., 2016](#)). 1,2,3-benzenetriol is identified from extract of *Eugenia flocossa* leaves ([Kala et al., 2012](#)). Eugenol spectrum is reported by [Li et al., \(2018\)](#) from food samples (coca cola). Diazepam, nordazepam and oxazepam are used from blood and urine samples using benzodiazepines assay ([Wallace et al., 1980](#)). Thiabendazole chromatogram is resulted from grapefruit extract analysis ([Schachterle, 2010](#)). Testosterone chromatogram is used from the report of [Fitzgerald et al., \(2010\)](#) analysis on human serum.

3. RESULTS AND DISCUSSION

3.1 GC/MS Interpretation of Simple Mixtures

3.1.1 Sample 1: Carbon disulfides

First mixture sample is the simplest example, carbon disulfides (**Figure 8**). The mixtures are consisted of carbon disulfide (CS₂) and other minor compounds. [Mujawar et al., \(2014\)](#) reported that CS₂ analysis could be used to determine the concentration of dithiocarbamate pesticide in a mixture. **Figure 9** shows GC/MS chromatogram of CS₂.

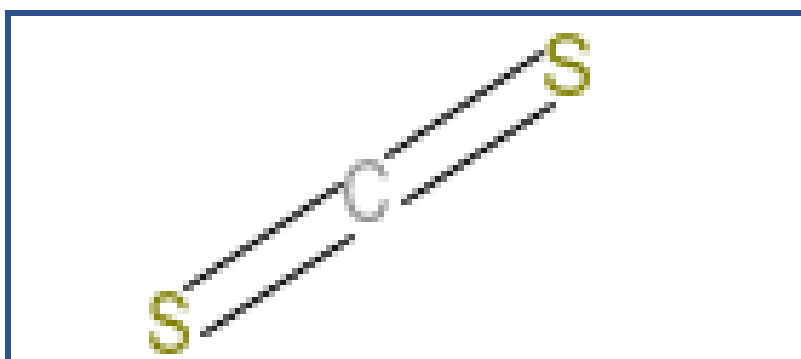


Figure 8. CS₂ structure.

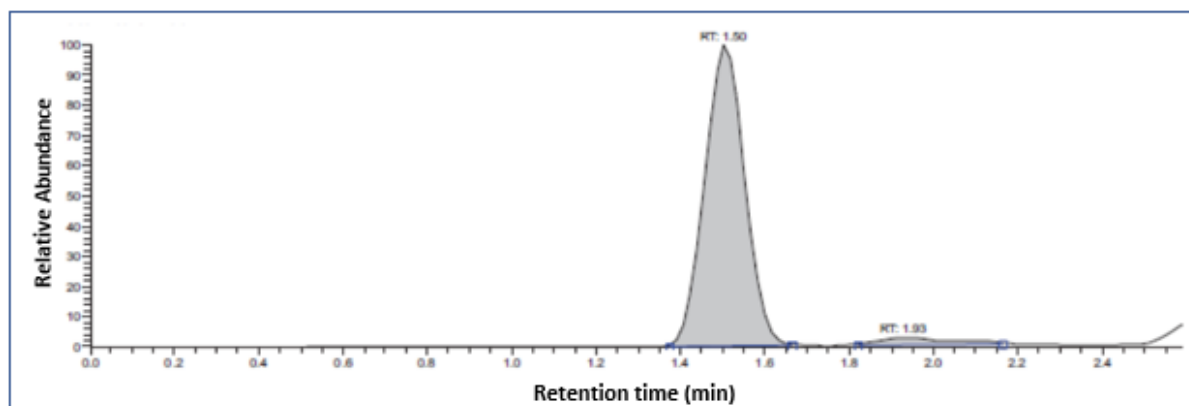


Figure 6. Chromatogram of CS₂ (Mujawar *et al.*, 2014).

Using step-by-step procedure to read and interpret the chromatogram, we can analyze that:

1. **Step 1:** Identify the peaks display. Based on the chromatogram display, observed that the sample has been ideally separated with a huge difference of area. There are two analytes in total detected after separation of the sample by the column.
2. **Step 2:** Identify the order of the peaks along the RI axis. First detected compound is CS₂ at RI 1.5 min. Whereas at RI of 1.93 min; other compounds is observed.

Figure 10 represents the mass spectrum of CS₂ individual compound. According to the procedure, the interpretation steps are as follow:

- 1) **Step 1:** Identify the molecular ion. The molecular ion is observed in the rightest bar. Its m/z value is 76, similar with its molecular weight.
- 2) **Step 2:** Identify the fragmentation pattern. Based on the bar graph, there are two other peaks besides the molecular ion. The peak is at m/z 76, 44, and 32. Due to its simple structure and the atoms built it, we can simply know that the logical mass losses are from S and C as the masses are identical with their atomic weight.
- 3) **Step 3:** Determine for significant ion series. As the structure is the simplest one, none of the series will be observed.
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). The molecular ion isotope pattern of CS₂ has similar occurrence with typical pattern of S₂. Its relative intensity represents the proportional comparison between ³²S and ³⁴S as illustrated in **Figure 11**.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. Both cleavages happen between C and S.

Using the step-by-step procedure, we can identify conclusively that based on the mass spectra interpretation, the compound at relative RI 1.5 is CS₂.

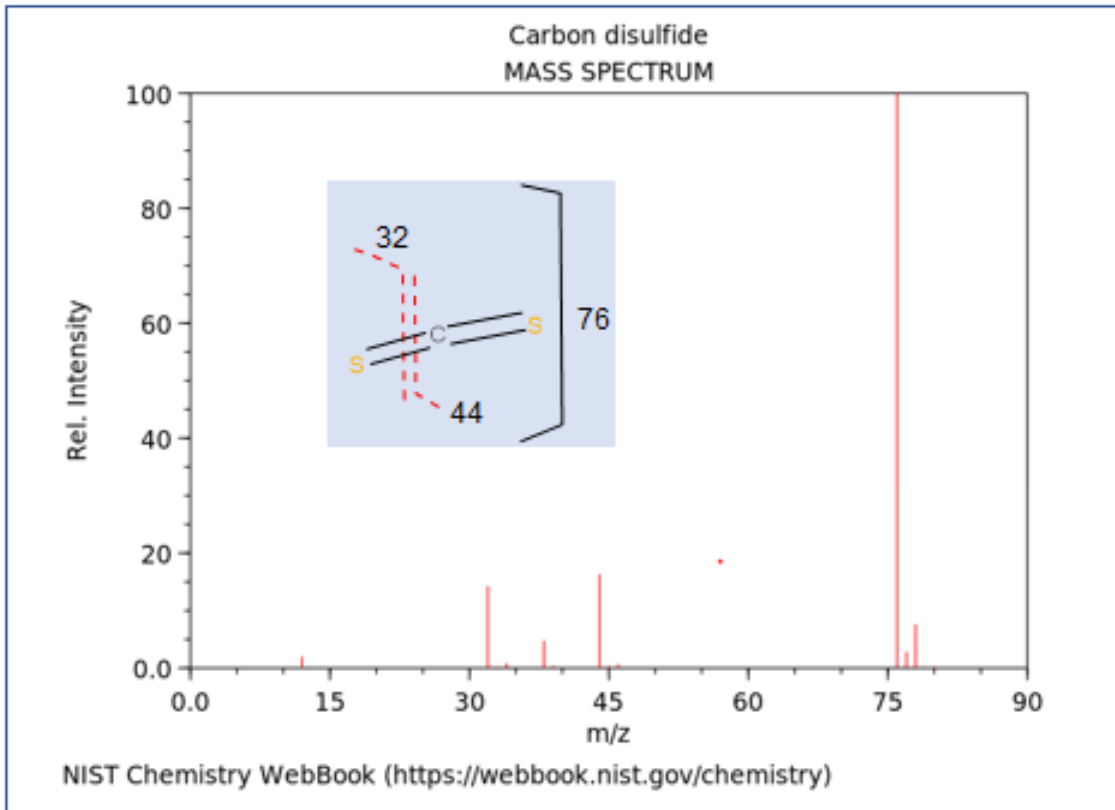


Figure 7. CS₂ mass spectrum and its interpretation result using ab initio procedural steps.

Probability of the occurrence of a ³⁴ S atom [%] – M+2		
S ₁	0.79	
S ₂	1.58	
S ₃	2.37	

Figure 8. Relative intensities of the A+2 signals of Sulphur (³⁴S) by increasing atom numbers (Schwarzbauer & Jovančićević, 2020).

3.1.2 Sample 2: Ethanol and acetaldehyde

Second mixture sample is relatively simple, consisted of three major organic compounds, namely ethanol, acetaldehyde, and toluene. The research conducted by Heit *et al.*, (2017) was purposed to identify ethanol and acetaldehyde as alcohol addiction cause metabolites in the living tissues. **Figure 12** shows GC/MS chromatogram resulting.

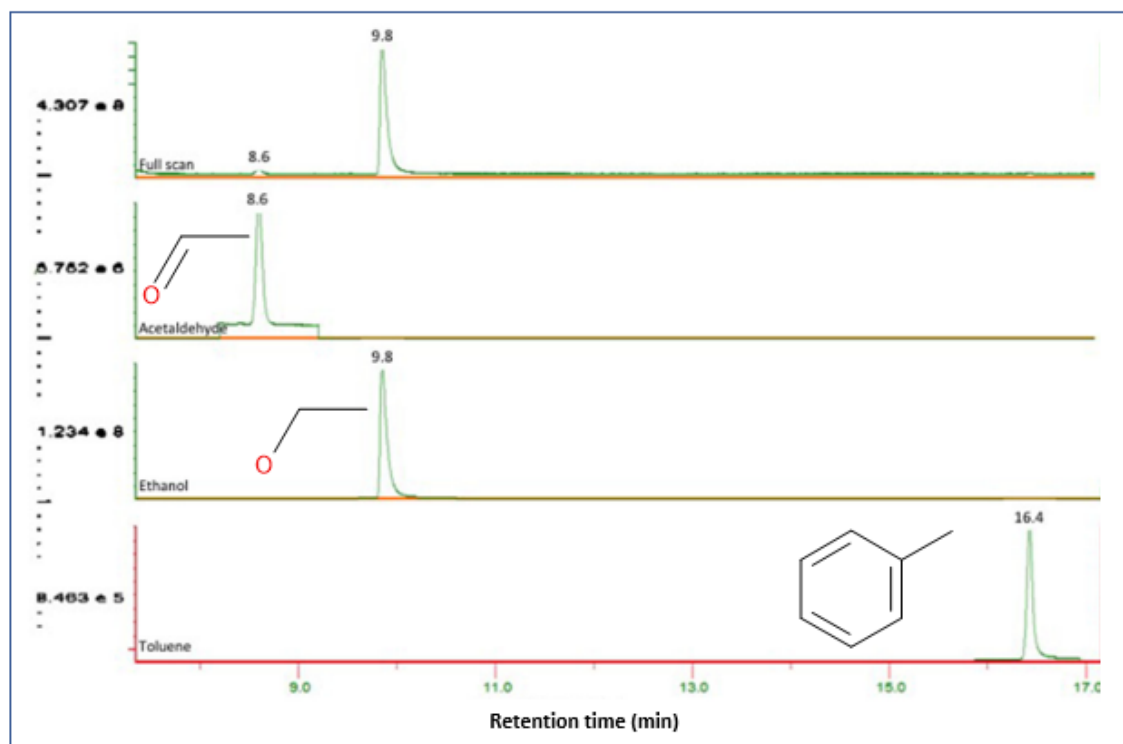


Figure 9. Mixture and individual chromatogram of acetaldehyde, ethanol, and toluene (Heit *et al.*, 2017).

Using step-by-step procedure to read and interpret the chromatogram, we can analyze the step as follow:

- 1) **Step 1:** Identify the peaks display. Based on the chromatogram display, observed that the sample achieved ideal separation with a distinct resolution. In particular, ethanol was detected sharp and strong.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. First detected compound is acetaldehyde at RI of 8.6 min; ethanol firmly observed at RI 9.8 min; while toluene peak rises at 16.4 min.

On the other hand, relative spectral mass is to read and interpret by the following step-by-step procedure:

- 1) **Step 1:** Identify the molecular ion. Based on **Figure 13**, molecular ion of ethanol is measured by 45.1 m/z, while in the **Figure 14**, molecular ion of acetaldehyde appears at 44.1m/z.
- 2) **Step 2:** Identify the fragmentation pattern. In **Figure 13**, the fragmentation pattern observed of m/z ratio is 45.1, 31.2, and so on. According to mass lost sequence, we assume that in logical order of the lost due to the removal of 1 methyl. It is indicative as the mass is 14 m/z. While for acetaldehyde (**Figure 14**), the mass loss represented by m/z order of 44.1 (molecular ion) to 29.2 (base peak). The logical order of mass loss is due to 1CH₃.
- 3) **Step 3:** Determine for significant ion series. Mass losses from ethanol and acetaldehyde are significant series of aliphatic chain as described in **Table 1**. The residual fragments also typical for starting mass of alcohols and aldehyde groups.
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). There is no S, Cl, nor Br containing.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. As the structures are relatively simple, the cleavages are following the series pattern.

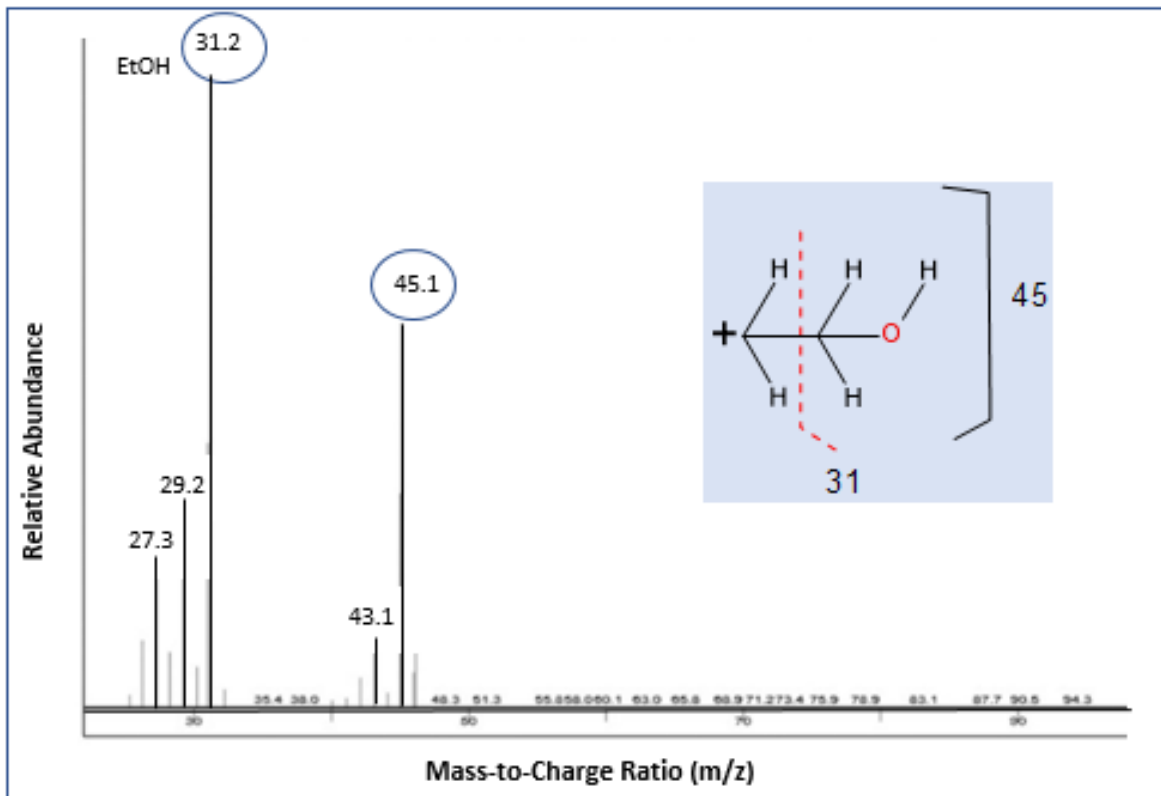


Figure 10. Selected mass spectrum of ethanol (Heit *et al.*, 2017), and its interpretation result.

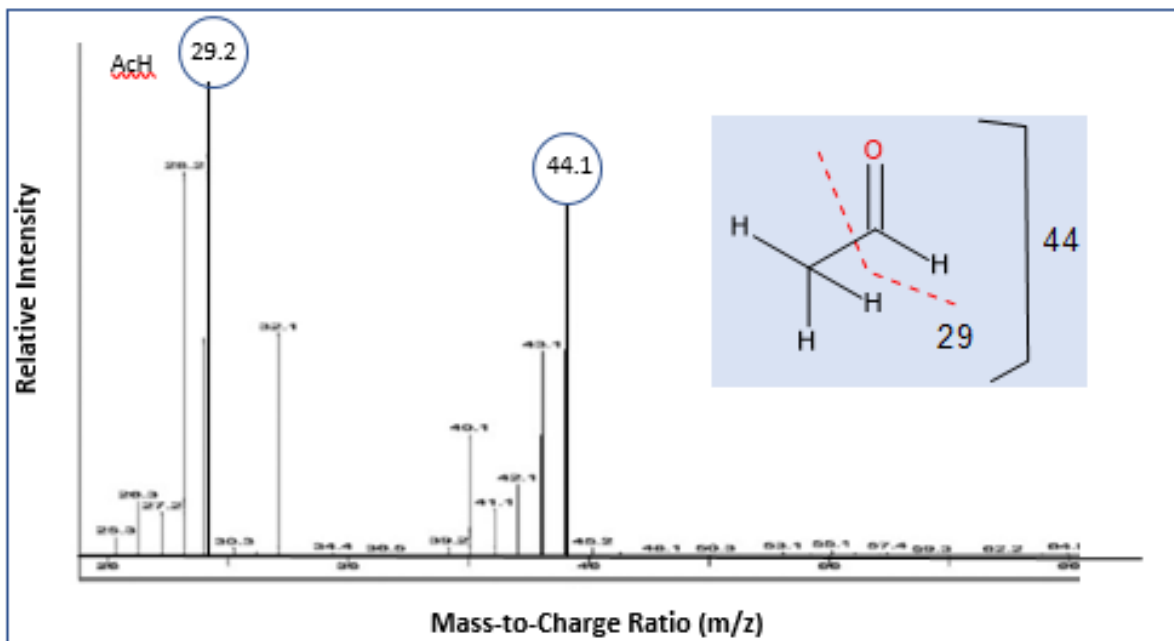


Figure 11 Selected mass spectrum of acetaldehyde and its interpretation result.

3.1.3 Sample 3: Dichloro(silyloxy)silane

Sample 3 is dichloro(silyloxy)silane. It is not organic compound as GC/MS mostly used. However, the application of the technique is possible to identify the presence of impurities in the mixtures, especially for qualitative analysis after coupled with ICP (inductively coupled plasma). Originally the compound has molecular formula $\text{Si}_2\text{Cl}_2\text{OH}_4$. Thus, the qualitative information based on the spectra is expected to give the predicted possible structure of the compounds (whether it has structure of dichlorosilane or not), besides to identify some impurities existence. **Figure 15** shows GC/MS analysis spectra of dichloro(silyloxy)silane ($\text{Si}_2\text{Cl}_2\text{OH}_4$), with its interpretation using procedural steps.

The interpretation of $\text{Si}_2\text{Cl}_2\text{OH}_4$ mixture chromatogram using step-by-step procedure is as follow:

- 1) **Step 1:** Identify the peaks display. Based on the GC/MS chromatogram in **Figure 15**, the spectrum shows separation processes are successful.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. The assumed trichlorosilane (or dichloro(silyloxy)silane, to be precise) compound is observed at relative retention time 8 min. The tailing effect next to assumed $\text{Si}_2\text{Cl}_2\text{OH}_4$ apparently comes from unknown component, which its peak comes up in RI 8.92 min. It gives a relatively higher peak as the possible concentration also fairly high.

The mass spectra step-by-step interpretation procedures are following:

- 1) **Step 1:** Identify the molecular ion. The first bar on the right side is observed at m/z 147 given by isotope peak. While the molecular ion peak is identical with the molecular weight of $\text{Si}_2\text{Cl}_2\text{OH}_4$ ion, 145.
- 2) **Step 2:** Identify the fragmentation pattern. In **Figure 15**, the fragmentation pattern observed of m/z ratio is 145, 109 (± 35.5), and 72 (-37). The pattern is closed to two times of chlore atoms released. According to mass lose sequence, we assume that in logical order of the lost due to the removal of first (^{35}Cl) and second (^{37}Cl) -Cl fragmented atoms.
- 3) **Step 3:** Determine for significant ion series. There is no ion series observed for no hydrocarbon containing in the structure.
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). From the fragmentation pattern in the step 2, we assumed that the isotopes are released. We can confirm from the isotope pattern in the molecular ion bars shape. The pattern is similar with the Cl_2 isotopes pattern in **Figure 7**. Hence, it is confirming the Cl_2 fragments in step 2.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. As the structures are relatively simple, the cleavages observed are only due to chlorines without another backbone fragments release following.

After the GC-MS spectra interpretation by introduced procedural steps, we can conclude that the compounds are identified as dichloro(silyloxy)silane ($\text{Si}_2\text{Cl}_2\text{OH}_4$) with dichlorosilane (SiCl_2H -) structure in the mixture.

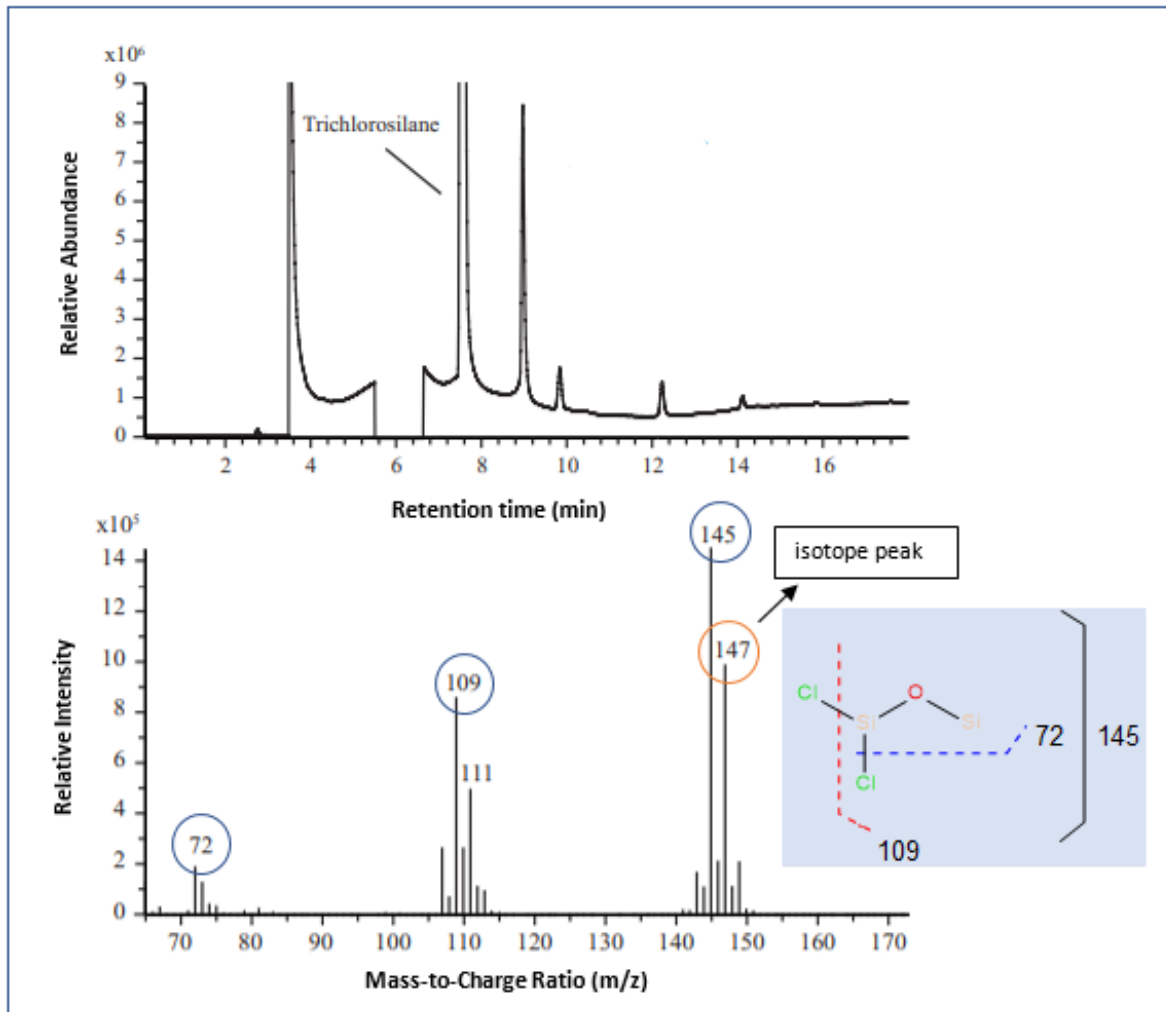


Figure 15. GC/MS analysis spectra of dichloro(silyloxy)silane ($\text{Si}_2\text{Cl}_2\text{O}_4$) (M.Geiger & McElmurry, 2020), with its interpretation using procedural steps.

3.2 GC/MS interpretation of fairly complex mixtures

3.2.1 Sample 1: Methyl-Tert-Butyl-Ether (MTBE)

First sample of fairly complex mixtures is methyl-tert-butyl ether in gasoline mixtures. Quach *et al.* (1998) reported their experiment to analysis MTBE and benzene in gasoline. Figure 16 shows chromatogram for gasoline mixtures in 1-chlorohexdecane. Gasoline was prepared from mixtures of MTBE, benzene, toluene and *o*-xylene.

Based on the ion chromatogram, the interpretation using step-by-step procedures are as follow:

- 1) **Step 1:** Identify the peaks display. Figure 16 represents the spectrum of gasoline mixture separation process. The complex pattern of chromatogram (multiple peaks) given by typical detection of large aromatic compounds; benzene, toluene, and *o*-xylene.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. Measurement of compounds masses indicate that MTBE ($m/z = 73$) tends to elute at the first place on relative RI 1.8 min (single peak). Benzene has more than one peak detected, 2.51, 8.57, and 9.52 min respectively. The same phenomenon shown by toluene and *o*-xylene chromatogram peaks, which have 4.58, 8.57, and 9.52 for toluene; 8.57 and 9.52 (both overlapped with benzene and toluene peaks).

The mass spectra step-by-step interpretation procedures are following:

- 1) **Step 1:** Identify the molecular ion. Individual mass spectrum of detected compound at 1.8 min is presented in **Figure 17**. The first bar (base peak) has m/z value 73. There is no molecular ion peak due to loss of a $-CH_3$ group during electronic impact ionization. It is the cause of only one peak in ion chromatogram shown, where the methyl group undetected in the molecular ion ($M_r = 88$).
- 2) **Step 2:** Identify the fragmentation pattern. In **Figure 15**, the fragmentation pattern observed of m/z ratio is 145, 109 (± 35.5), and 72 (-37). The pattern is closed to two times of chlore atoms released. According to mass lose sequence, we assume that in logical order of the lost due to the removal of first (^{35}Cl) and second (^{37}Cl) -Cl fragmented atoms.
- 3) **Step 3:** Determine for significant ion series. The first mass losses representing indicative aliphatic chain, so that we conclude that the leaving group is methyl ($-CH_3$). m/z value of 43 tends to be significant series of ethers group.
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). There is no S, Cl or Br in this mixture or structures.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. Besides methyl cleavage on methyl group, it is possible the cleavage on tert-butyl side resulting m/z value 31. However, no bar indicating such cleavage. m/z value 29 is closer to the cleavage of $-^+C_2H_5$. Hence, only one optional cleavage that most possible in this case.

Based on the GC-MS spectra interpretation by introduced procedural steps, we can conclude that the compounds are identified as MTBE from the mixture of gasoline.

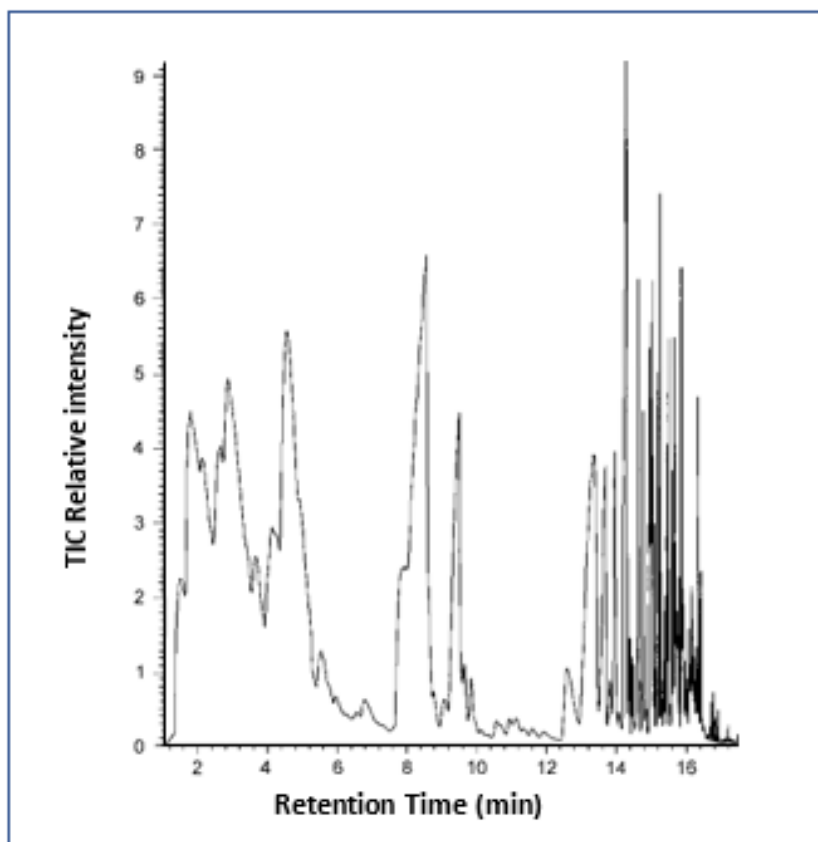


Figure 16. GC/MS chromatogram for the gasoline mixture sample in 1-chlorohexadecane.

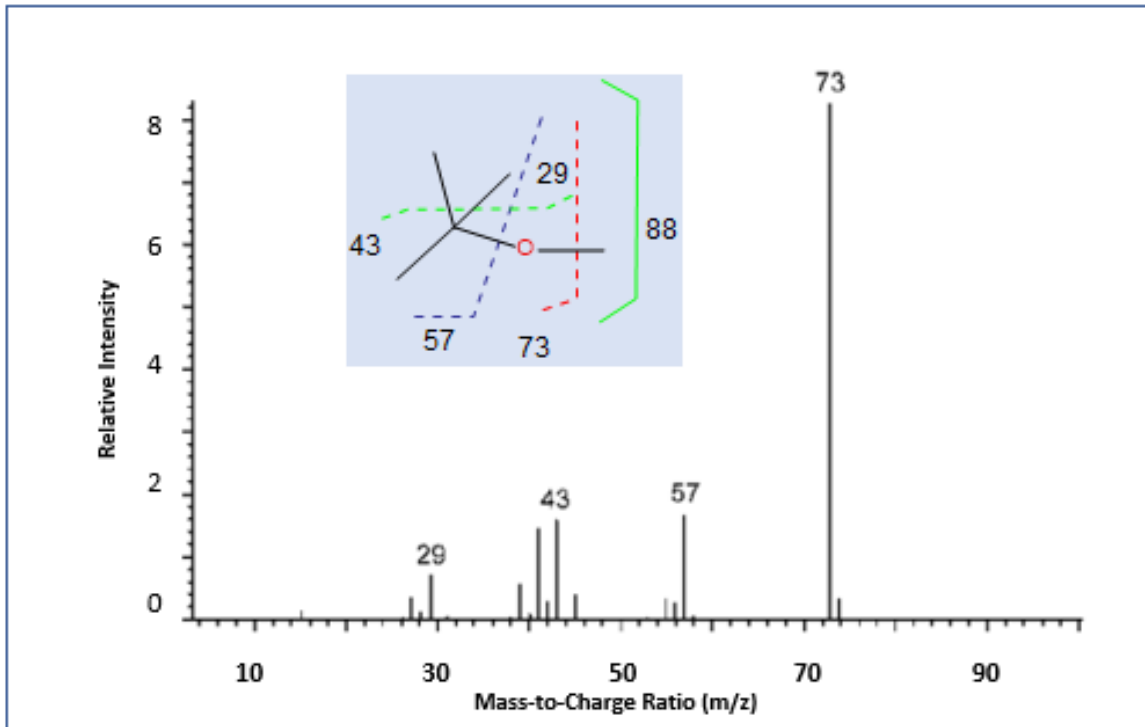


Figure 17. MTBE chromatogram (Quach *et al.*, 1998), with ab initio interpretation using procedural steps.

3.2.2 Sample 2: Methyl-Palmitic Ester and Methyl-Oleic Ester

The second samples to interpret and identify are methyl-palmitic ester and methyl-oleic ester in the tomato seed oil (TSO) mixture. Both are two compounds obtained from derivatized TSO fatty oil (Botinestean *et al.*, 2012). Their differences come from chain length and the presence of unsaturated bond, as shown in **Figure 18** and **Figure 19**.

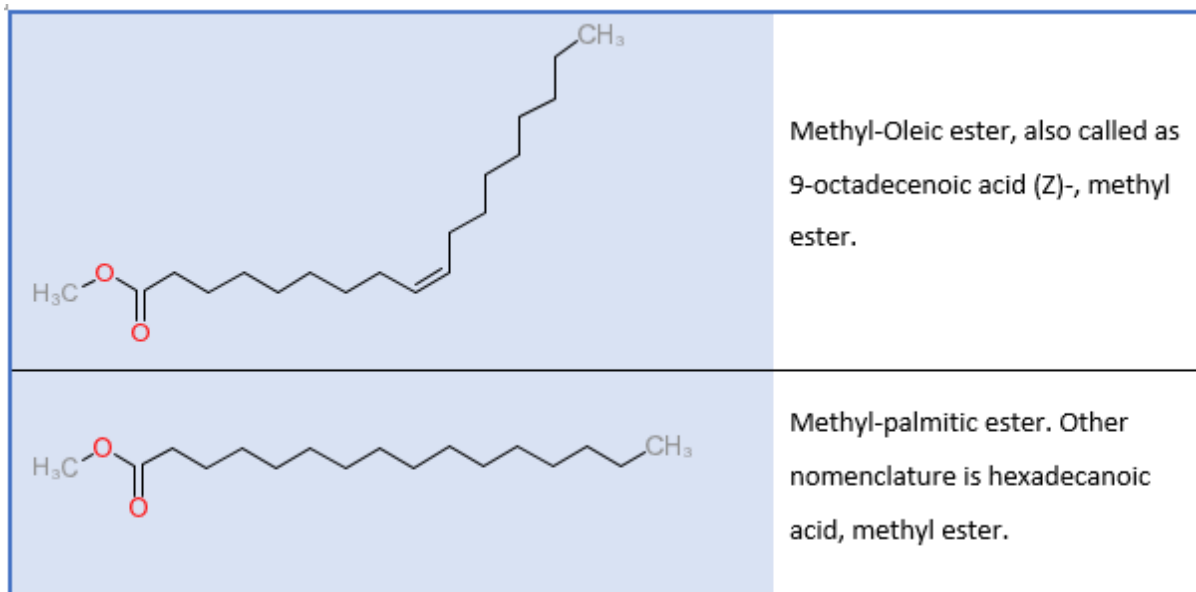


Figure 18. FAME structures of palmitic and oleic derivatized TSO.

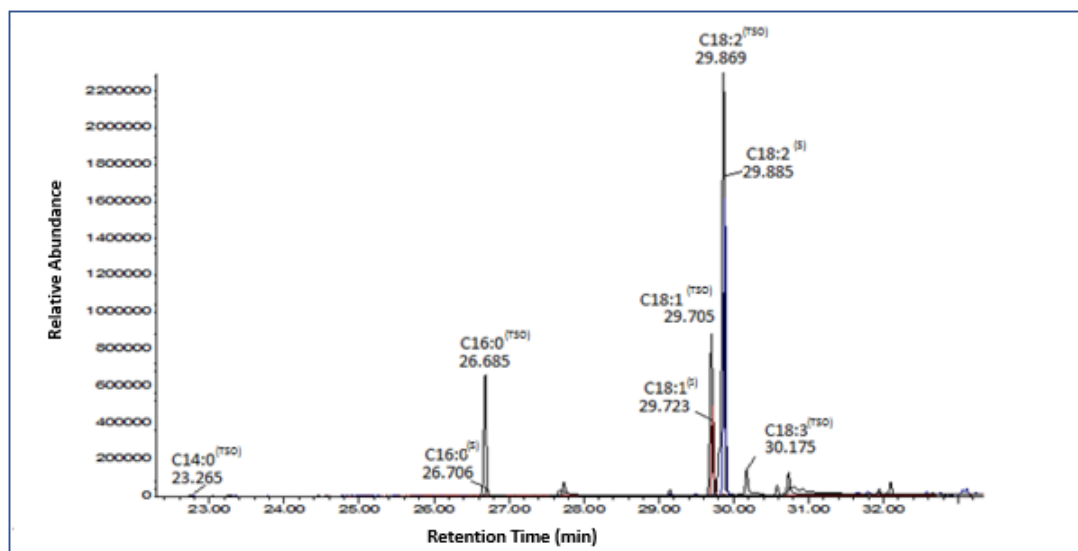


Figure 19. Chromatogram of Tomato Seed FAME (Botinestean *et al.*, 2012).

Based on chromatogram of TSO in **Figure 19**, we can interpret the spectrum using procedural steps:

- 1) **Step 1:** Identify the peaks display. **Figure 19** depicts that palmitic and oleic TSO FAME are separated from the mixture.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. Palmitic methyl ester is eluted at RI 26.685 min, while oleic derivate is measured at 29.705 min.

Since the main information has been withdrawn, the spectra of both compounds mass are interpreted individually by the procedural steps:

- 1) **Step 1:** Identify the molecular ion. Individual mass spectrum of methyl-palmitic ester in **Figure 20** illustrating the fragmentation pattern processes. Its molecular ion detected at m/z value 270. In the meantime, methyl-oleic ester molecular ion seen at m/z value 296 in **Figure 21**.

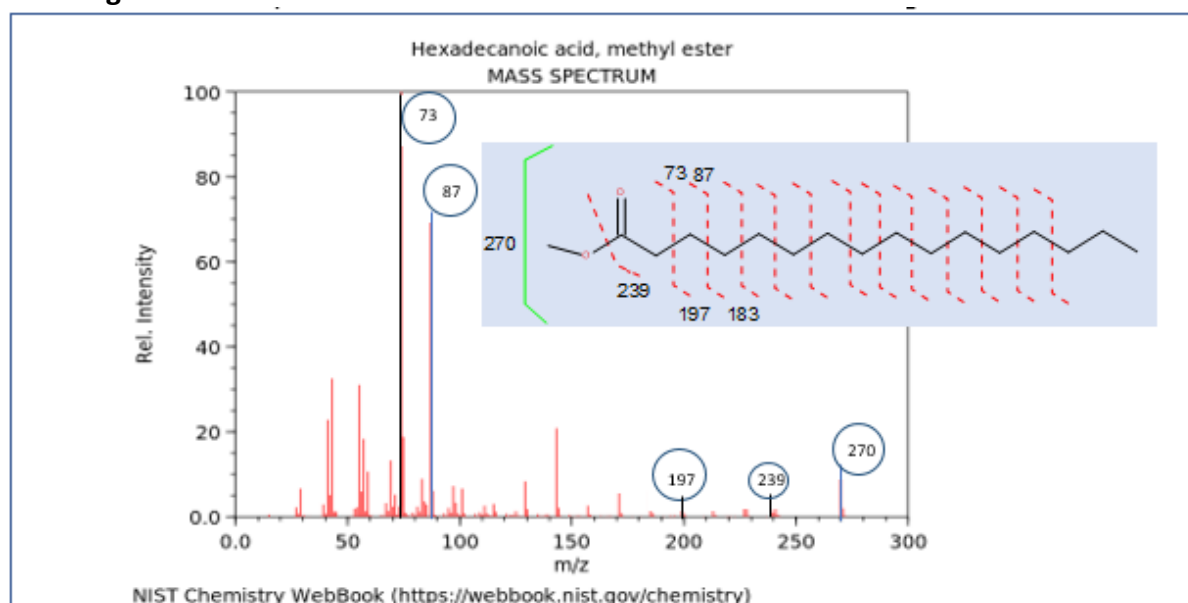


Figure 20. Palmitic FAME mass spectrum with ab initio interpretation using procedural steps.

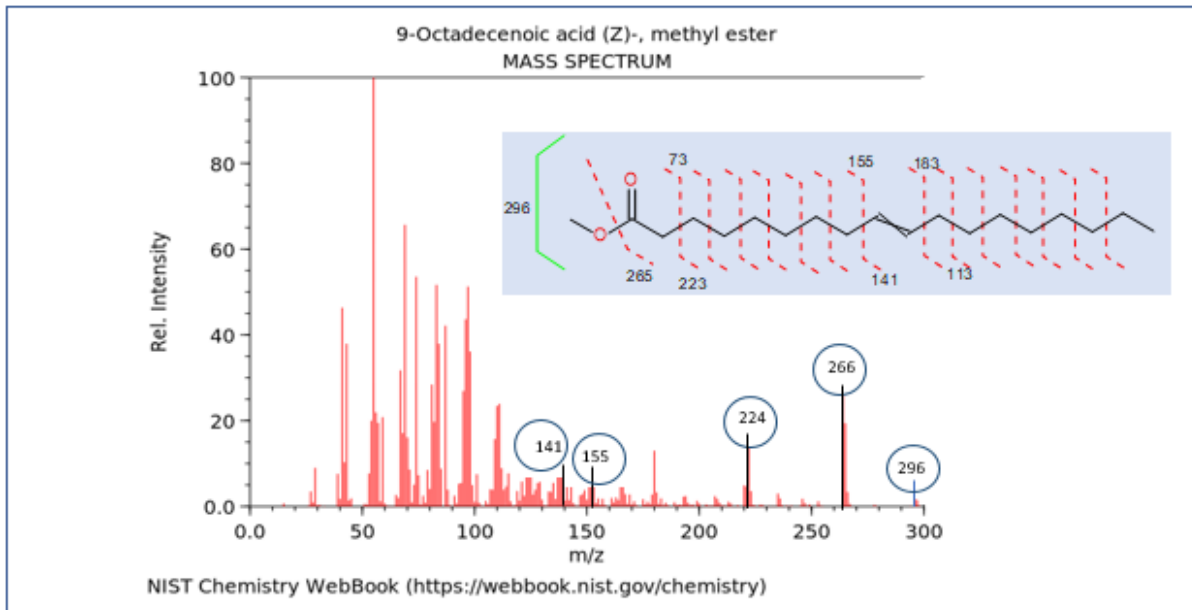


Figure 21. Oleic FAME mass spectrum with ab initio interpretation using procedural steps.

- 2) **Step 2:** Identify the fragmentation pattern. Both palmitic and oleic derivatives show the fragmented peaks of methoxy group as the first leaving fragment. The next fragments belong to the carbonyl group, whereas the aliphatic chain follows to break the bonds. The difference in fragmentation pattern seems due to the presence of a double bond in the octadecenoic chain, as shown in **Figure 21**.
- 3) **Step 3:** Determine for significant ion series. The fragmentation patterns of either palmitic or oleic show the significant series of aliphatic alkanes and starting mass of ether groups. The serial cleavages shown in **Figure 20** and **Figure 21**.
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). Both compounds have no S, Cl, nor Br in their structures.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. The cleavages could have happened in the methyl group to break its bond with oxygen. The spectrum, however, indicates the cleavage prefers the methoxy group to the methyl one.

Based on the GC-MS spectra interpretation by the introduced procedural steps, we can conclude that the compounds are identified as methyl-palmitic ester and methyl-oleic ester from the mixture of tomato seed oil (TSO).

3.2.3 Sample 4: 1-(2-Hydroxyethyl)-2-Imidazolidinone (HEIA)

The last one of the fairly mixture sample 5 is 2-aminoethylethanolamine (AEEA) compound degradation identification from post-combustion products mixtures (Saeed *et al.*, 2017). It has nitrogen in the structure of the compounds. The degradation product is 1-(2-Hydroxyethyl)-2-imidazolidinone (HEIA) as shown in **Figure 22**. **Figure 23** shows the gas chromatogram (top) and mass spectrum (bottom) of HEIA.

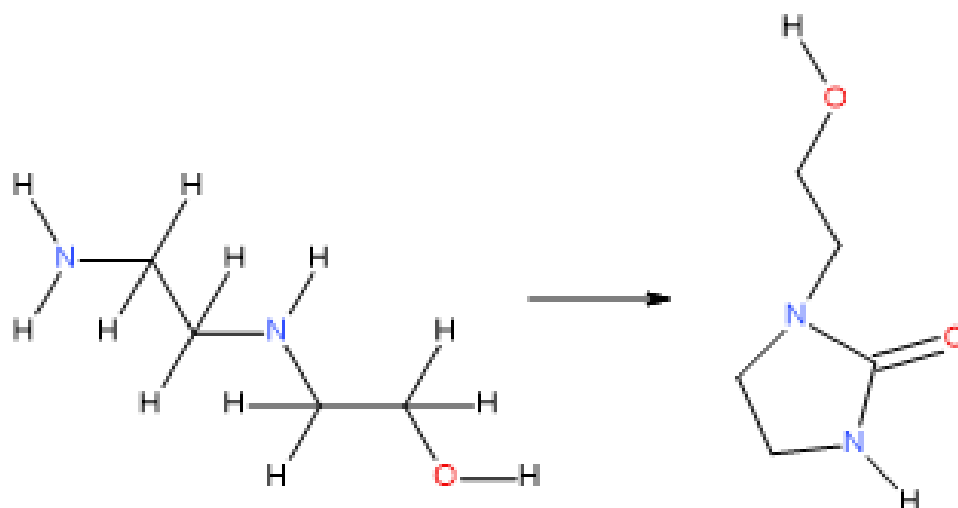


Figure 22. Chemical degradation reaction of AEEA to HEIA.

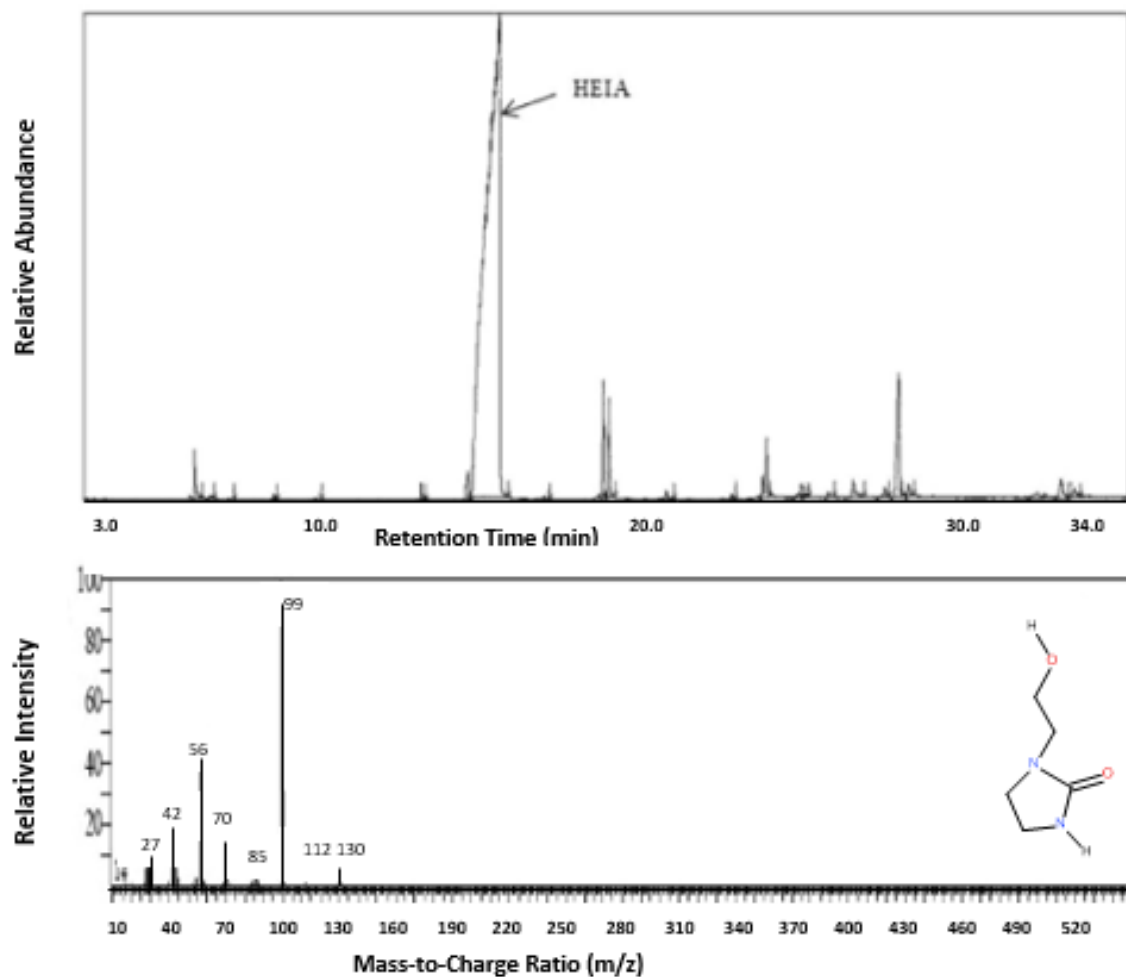


Figure 23. Gas chromatogram (top) and mass spectrum (bottom) of HEIA (Saeed *et al.*, 2017).

According to the case, we are identifying the spectra of HEIA formed in the reaction. The general step-by-step procedure are as follow:

- 1) **Step 1:** Identify the peaks display. **Figure 23** (top picture) shows some tiny peaks in generally. In contrary a single big and tall peak standing on the middle of the line. The highest and biggest peak represents HEIA produced and separated from the mixture components.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. As the major component resulted in the reaction and analyzed by the instrument, HEIA has retention time at 15 min. So, another compound on the left and right are neglected as they are not the focus of the study.

Based on **Figure 23**, we can summarize our study finding using step-by-step procedure below:

- 1) **Step 1:** Identify the molecular ion. **Figure 23** (bottom) shows the mass spectrum of HEIA. The first bar detected has m/z ratio 130, which is identic with relative molecular mass of HEIA.
- 2) **Step 2:** Identify the fragmentation pattern. The fragmented isotope detected subsequently are 99, 70, 56, and so on. The pattern is so close to the data fetched from NIST library as shown in **Figure 24**.
- 3) **Step 3:** Determine for significant ion series. The pattern of mass losses in **Figure 24** are indicative of alcohols ion series (31, 45, and so on).
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). There is no S, Cl, or Br in this structure.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. Possible cleavages of any bond in and out of the imidazolinone ring are evaluated. Mass fragments possible the cleavages before (aliphatic chain) and after the nitrogen from the ring involving its ring opening, and followed by subsequent fragmentations.

From the result of spectra interpretation, we conclude that HEIA is identified from the mixture of post-combustion product in RI 15 min.

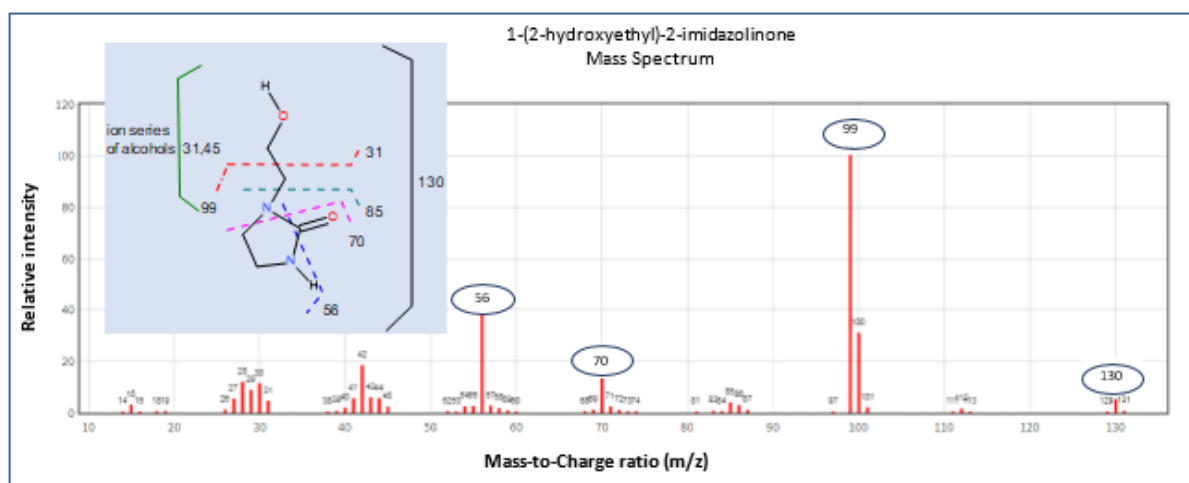


Figure 24. Mass spectrum of HEIA and its interpretation using procedural steps.

3.3 GC/MS Interpretation of More Complex Mixtures

3.3.1 Sample 1: β -cymene

First sample of more complex compound in this study is β -cymene (Figure 25), which is one of the VOCs compound component from Chinese traditional herbs (He *et al.*, 2016). Different traditional preparation methods resulted different aromatic components. The gas chromatogram from several preparation methods shown in Figure 26.

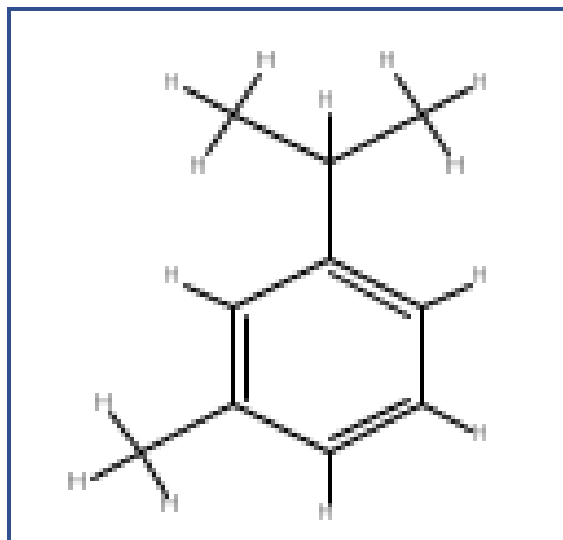


Figure 25. β -cymene structure.

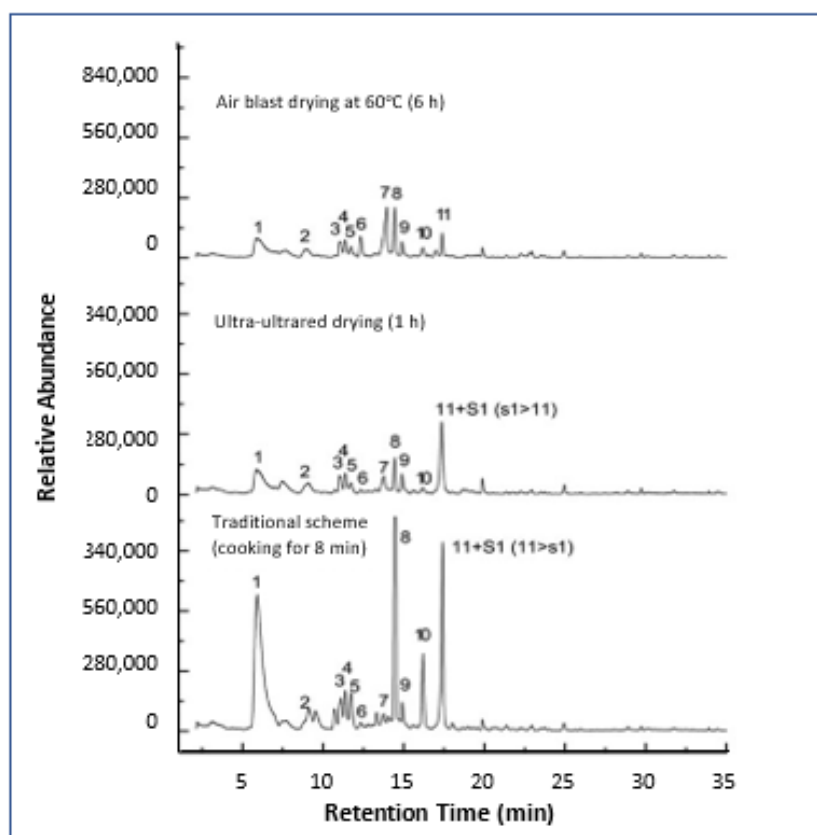


Figure 12. GC/MS comparison for the different honey-roasting products (segment 0-35 min).

Based on the chromatogram in **Figure 26**, we analyze the spectrum using step-by-step approach:

- 1) **Step 1:** Identify the peaks display. **Figure 26** shows that the sample has been separated by the column.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. The pattern of RI's order is in the very identical in three spectra for three sample. To interpret which is the peak of β -cymene, the common method is to compare with the references used the similar method of separation and column. This work has been reported in the article currently studied, based on the comparative references study it tends to come up at RT 9.4 min. So, the interpretation is continued to the individual mass spectra.

The mass spectra step-by-step interpretation are following:

- 1) **Step 1:** Identify the molecular ion. **Figure 27** represents the mass spectrum of assumed β -Cymene. First bar detects molecular ion at m/z value 134. It is similar with β -Cymene molecular mass. The value is successful to distinguish β -Cymene from another compound with has similar RI.
- 2) **Step 2:** Identify the fragmentation pattern. Based on mass spectrum, the fragments are detected at m/z value 119, 91, 77, and so on. Its first mass loss is due to methyl group for its indicative mass ($m/z = 15$). The second fragment also because of aliphatic chain (43). While the m/z 77 is typical for benzene mass spectrum. To simplify the interpretation, the benzene fragmentation will not be conducted further.
- 3) **Step 3:** Determine for significant ion series. The major losses are due to aliphatic chain as 15 and 43 are significant ion series of alkanes.
- 4) **Step 4:** Identify the isotope pattern (S, Cl, Br). There is no S, Cl, nor Br atom in the structure.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. There is no hetero atom in the β -Cymene compound structure.

Based on the step-by-step procedures, we conclude that the compound by which its RI is 9.41 and the spectrum interpreted is identified as β -Cymene.

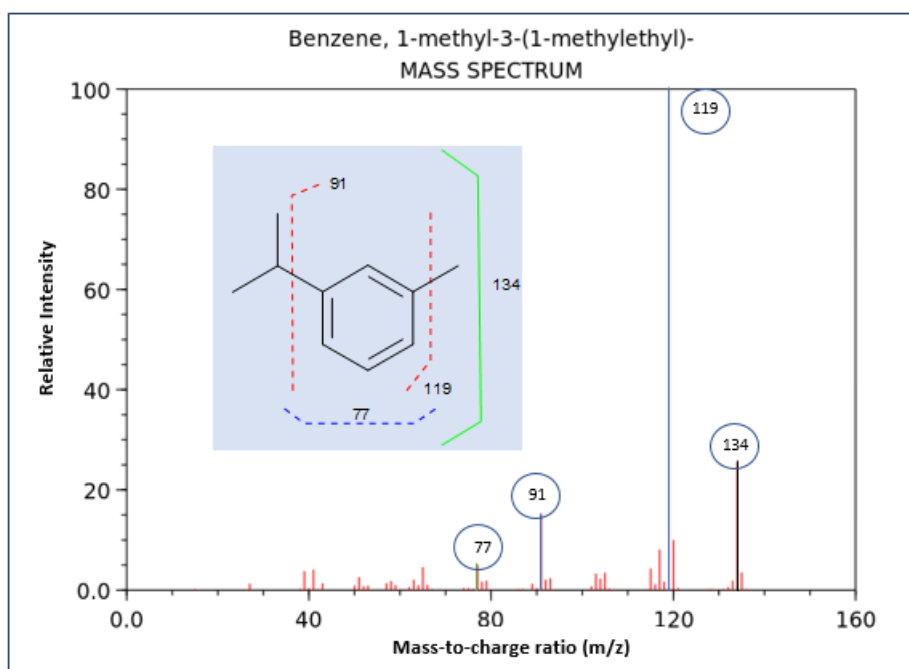


Figure 27. β -Cymene mass spectrum and its interpretation result using procedural steps.

3.3.2 Sample 2: 1,2,3-Benzenetriol

Sample 2 for more complex compound in organic mixture is 1,2,3-benzenetriol *Eugenia floccosa* leaves (Kala *et al.*, 2012). It has relatively complicated chromatogram pattern as well as tends to more complex mixture characteristic. **Figure 28** shows the overlapped and broaden TIC peaks appearance.

The step-by-step interpretation procedure are as follow:

- 1) **Step 1:** Identify the peaks display. **Figure 28** has plethora of peaks, which means separated components are detected. The figure also displays imposed data of relative retention indices with relative molecular masses. 1,2,3-benzenetriol compound has molecular mass 126 gr/mol.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. The data of MR gives us a hint to narrow the identification. We focus on the peaks that show molecule masses 126 gr/mol. Unfortunately, there are more than one peak detected to have similar number in different retention time.

Based on the mass spectra step-by-step interpretation, some explanation come up as follow:

- 1) **Step 1:** Identify the molecular ion. **Figure 29** depicts the mass spectrum of 1,2,3-benzenetriol. The first right and highest bar belongs to 1,2,3-benzenetriol molecular ion with m/z value 126.
- 2) **Step 2:** Identify the fragmentation pattern. Base peak is observed at 108 of m/z value, followed by some 97, 80, and so on. First fragment ($m/z=108$) has been expected for the first -OH group leaves at first. However, the second fragment is too high by m/z value as what to expect will be about 92. While peak of 80 is a bit higher as well than what is believed belongs to benzene fragment pattern should be at 77 or 78, according to logical order of mass losses of 3 -OH groups leaving the ring one-by-one.
- 3) **Step 3:** Determine for significant ion series. Indicative ion series can be guessed easily for -OH groups as its mass are reduced by 18 value of m/z . Since the structure has three hydroxyls, the bonds breaking needs three times of mass deduction in the spectrum. However, the number of mass ratio is too higher than what expected by calculation.
- 4) **Step 4:** Identify isotope pattern. There is no S, Cl, nor Br atom exists in the structure, but when we analyze the isotope peaks pattern, it gives an interesting information. The m/z value from the second fragment onward are dominated by isotope peaks. That is why the m/z value tend to be higher than it used to be. ^{13}C isotopes make their relative intensities higher than ^{12}C after first -OH group leaves the benzene ring. The resulting m/z gap with the ^{12}C -benzene with two hydroxyl groups is higher than one or three of it attached to the benzene. It is shown from its relative intensity is lower than the other two. The last and basic benzene fragment without any -OH group shows narrower gap with ^{12}C -benzene ($m/z=77$), so that it confirms its stability for this favorable structure compared to the prior ones.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. There is no another hetero atom than in hydroxyl leaving group. Besides some rational isotope peaks of ^{13}C with dominating m/z value of the benzene, no other cleavages noticed out of the peak.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as 1,2,3-benzenetriol. The illustration of the spectrum interpretation is shown in **Figure 29**.

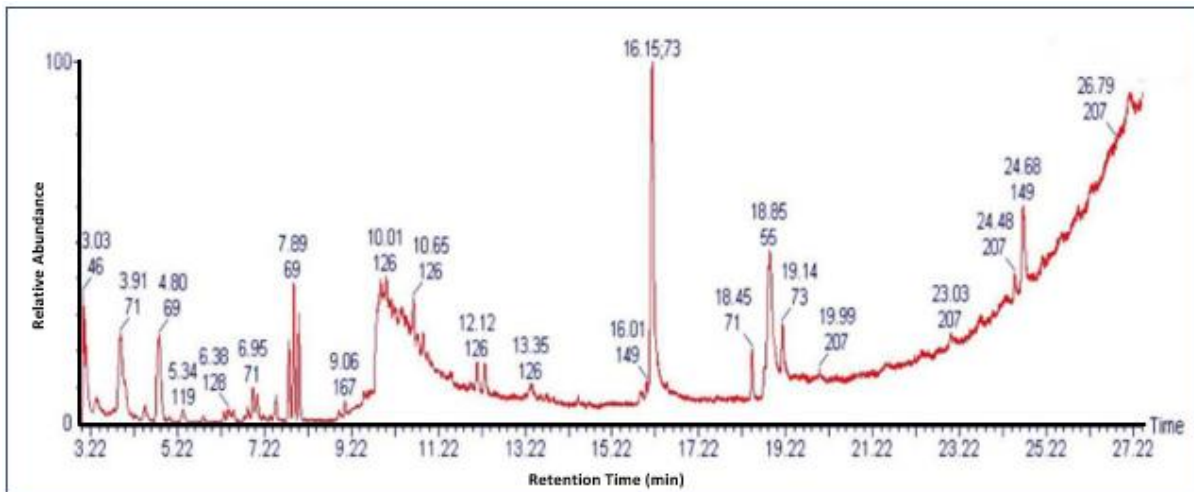


Figure 28. Chromatogram of the ethanolic leaf extract of *Eugenia flocossa* (Kala et al., 2012).

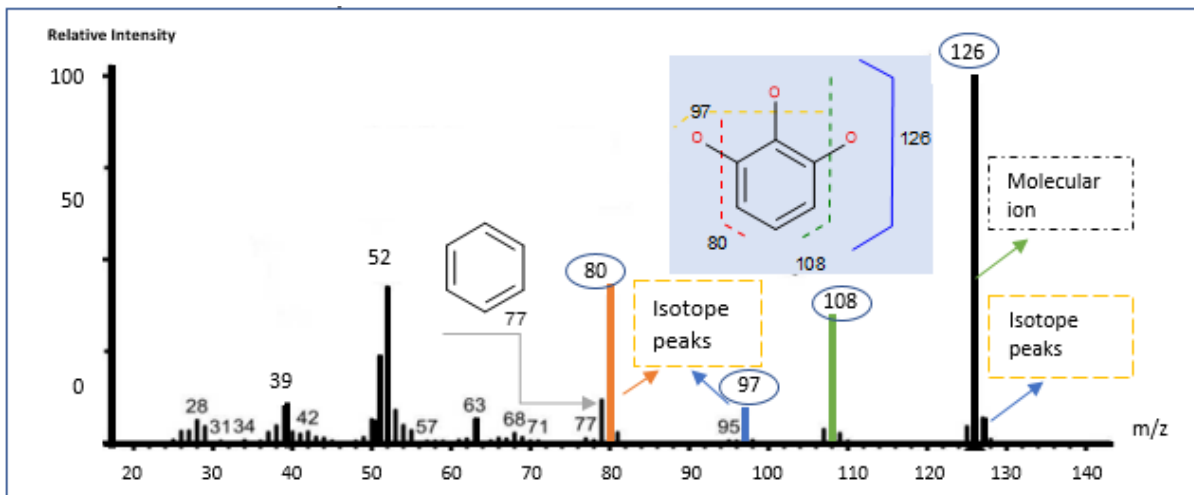


Figure 29. Mass spectrum of 1,2,3-benzenetriol and its interpretation via ab initio procedural steps.

3.3.3 Sample 3: Eugenol

Sample 3 of more complex compound in organic mixture for GC/MS spectra determination is eugenol (**Figure 30**). The compounds were analyzed from foods and beverages (Li et al., 2018). In this study, the chromatogram chosen as the sample is from coca cola mixtures. The analyzed chromatogram uses comparative line with internal standard. The stepwise procedure to interpret the chromatogram as follow:

- Step 1:** Identify the peaks display. **Figure 31** shows multiple peaks detected, in which a single dominating peak distinguished from another separated component. Based on cooperation result with internal standard mixtures, identified that eugenol retained at RI 9.3 min.
- Step 2:** Identify the order of the peaks along the RI axis. The chromatogram also shows another 'smooth' low peak at RI around 8.7 min. The remaining peaks are too low to observe, so we cannot conclude anything from them.

Based on the mass spectra of eugenol in **Figure 32**, the interpretation according stepwise procedure are as follow:

- 1) **Step 1:** Identify the molecular ion. **Figure 32** illustrate the mass spectrum of eugenol. The first detected on the right side appears not from eugenol, as eugenol has molecular mass around 164m/z. So, it will be excluded from structural fragments identification. The next m/z bar belongs to the eugenol molecule for its value is 163 closes to eugenol molecular mass.
- 2) **Step 2:** Identify the fragmentation pattern. Base peak is observed at 147 of m/z value, followed by some 135, 107, and 91. First fragment (m/z= 147) is expected for the first -OH group leaves the molecule structure. The second fragment is significant for alcohol ion series, as the mass losses are relatively similar to ethanol fragment. The alkyl group tends to remove resulting the fragment with m/z 107, following the elimination of -OH group in the first place. While the last one, given by phenolic fragment based on the simulation of relative residual fragmentation tool.
- 3) **Step 3:** Determine for significant ion series. As mentioned above, the fragmentation patterns show very indicative significant ion series of alcohol and alkanes groups according to the mass loss patterns. Indicative ion series can be guessed easily for -OH groups as its mass are reduced by 18 value of m/z.
- 4) **Step 4:** Identify isotope pattern. The spectrum in **Figure 32** has no isotope peaks to interpret. Hence, some inaccurate pattern of mass losses cannot be discussed further. Nor any S, Cl, and Br atom exists in the structure.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. There is only one possible hetero atom cleavage in eugenol structure, that is by methoxy group which leaves the structure as alcohols as detected in the spectrum.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as eugenol. The chromatogram also in consistent with the individual fragments pattern in mass spectrum analysis.

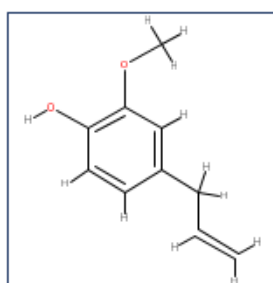


Figure 13. Eugenol structure.

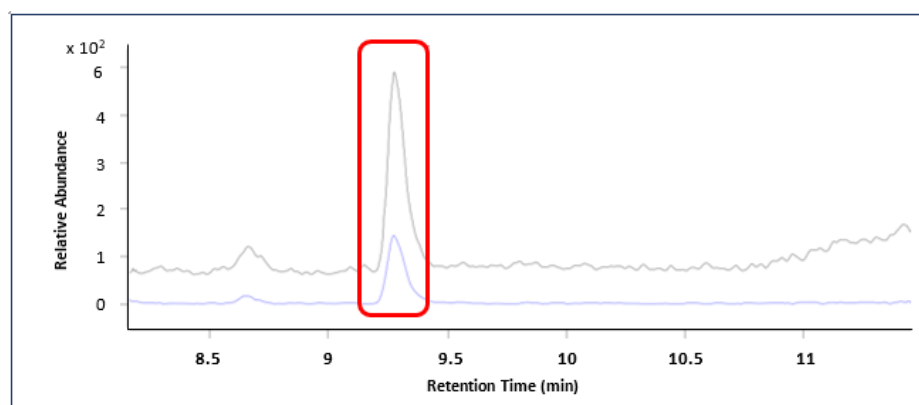


Figure 14. Ion chromatogram of eugenol sample in coca cola mixture (Li et al., 2018).

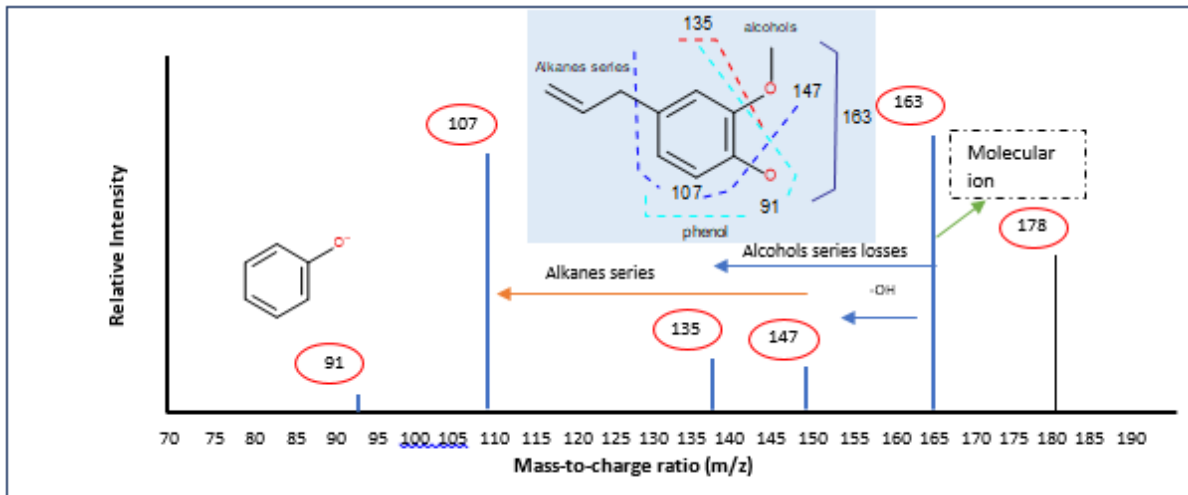


Figure 15. Mass spectrum of eugenol and its interpretation using ab initio procedure (Li *et al.*, 2018)

3.4 GC/MS Interpretation of very complex mixtures

The samples used for very complex compounds category are benzodiazepine derivatives (diazepam, nordazepam, and oxazepam), benzimidazole derivative (thiabendazole), and testosterone (underived). Diazepam, nordazepam and oxazepam are analyzed from the same mixture using benzodiazepines assay from human excreted like blood and urine (Wallace *et al.*, 1980). **Figure 33** illustrate the relationship of our samples with basic structure of benzodiazepine.

The mixture of benzodiazepine derivatives is analyzed using GC/MS. The chromatogram result is shown in **Figure 34**. Based on the spectrum, we can interpret it using the stepwise procedure:

- 1) **Step 1:** Identify the peaks display. In benzodiazepine assay conducted, the sample was compared with individual standard chromatogram. Thus, we have some conclusive identification of the chromatogram peaks. The separation is well proceeded and all peaks resulted are smooth and sharp.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. It is well informed that diazepam, nordazepam, and oxazepam peaks appear in RI 5.26, 5.49. and 6.29 min, respectively. Another peaks of benzodiazepine derivatives are also detected as well, but not included in the discussion.

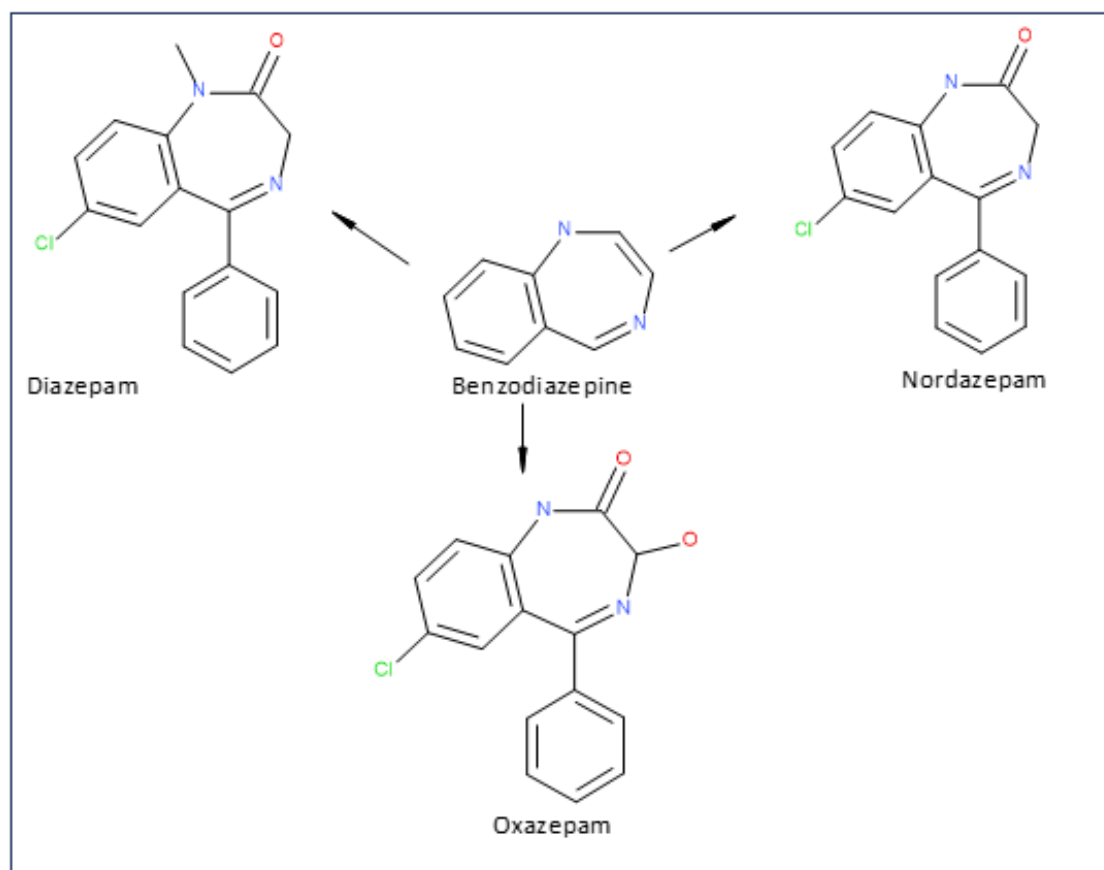


Figure 33. Schematic derivatives of benzodiazepines.

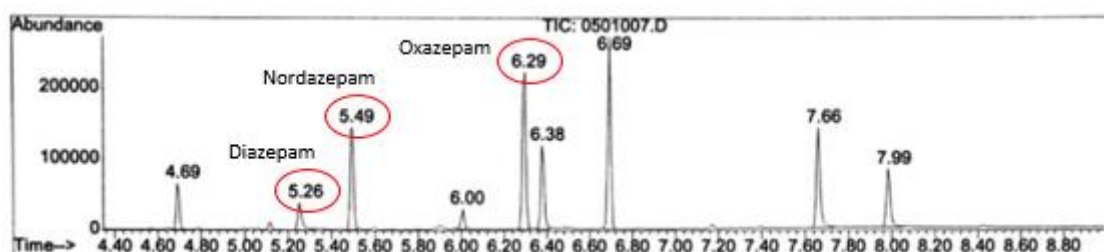


Figure 34. Chromatogram spectrum of human blood sample for benzodiazepine assay.

3.4.1 Sample 1: Diazepam

After the separation process conducted, the compounds individual mass spectra are about to identify. We make assessment on the first retained component. It is assumed as diazepam. To determine its structure, we do spectrum interpretation using step-by-step procedure consistently. The spectrum of diazepam is shown in **Figure 35**. Following are interpretation steps:

- 1) **Step 1:** Identify the molecular ion. **Figure 35** represents the mass spectrum of diazepam. First bar detected is identical to diazepam molecular mass, 284 m/z. Its nominal mass has an even number, follows nitrogen rule.
- 2) **Step 2:** Identify the fragmentation pattern. Next to the molecular bar is the base peak observed at 254 of m/z value, followed by some peaks at 249, 221, 199, and 165. For simple and easy interpretation of the compound, we do not discuss every single fragment we have. We can pick some noticeable and distinctive patterns only.

- 3) **Step 3:** Determine for significant ion series. As the base peak is detected at 254 m/z value, we can easily refer the lose mass as typical for ketones series (m/z is 30). The only -Cl in the structure is fragmented resulting mass reduction from 284 to 249 (-35 m/z). The alternative is, after ketones group leaves, -Cl follows them. The indicative peak appears at m/z 221 to support this assumption.
- 4) **Step 4:** Identify isotope pattern. Since diazepam has one Cl, its isotope pattern can be simply distinguished as we see in **Figure 7** of Cl isotopes pattern and its relative intensity. The fragment in spectrum of 249 m/z show typical isotope bar for one Cl.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. Any cleavages in the structure due to its nitrogen containing is considerable. **Figure 35** show some most probable cleavages close to electronegative hetero atom (i.e., nitrogen). Overall, there are three cleavage sites, next to nitrogen atoms in the diazepine ring. For illustration details, we provide m/z values of the fragments interpretation result, thus better comprehension can be achieved toward available figures of the mass spectra.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as diazepam. The compared chromatogram result is in line with the individual fragments pattern in mass spectrum analysis.

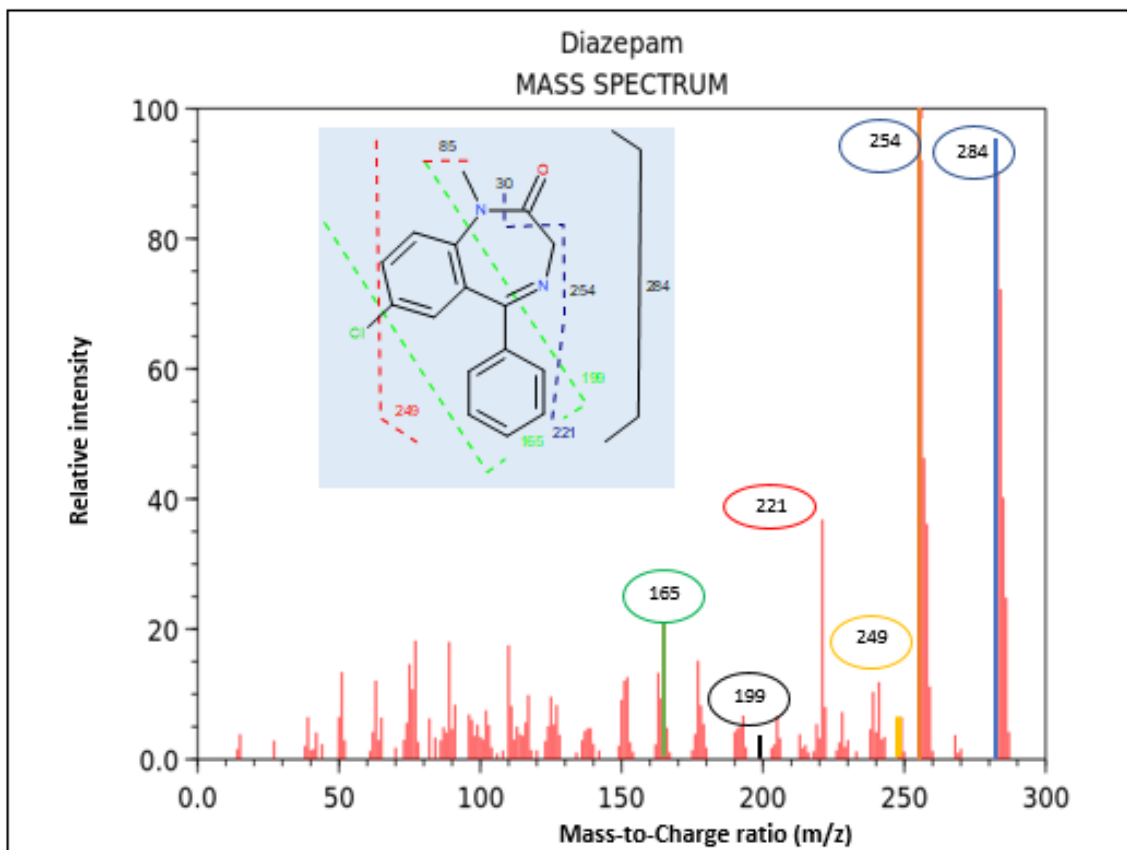


Figure 16. Mass spectrum of diazepam and its interpretation result.

3.4.2 Sample 2: Nordazepam

Second sample of very complex compound is nordazepam, another derivative of benzodiazepine. It is separated from the mixture of human serum, detected at RI 5.49 min over benzodiazepines assay method. Based on its individual mass spectrum in **Figure 36**, we can interpret it and identify the compound using step-by-step procedure as follow:

- 1) **Step 1:** Identify the molecular ion. For the nordazepam individual mass spectrum, we have a peak at m/z 270. It is similar to nordazepam molecular mass. As seen in **Figure 36**, the molecular ion has the strongest intensity. The compound has shown that its nominal mass has an even number, follows nitrogen rule.
- 2) **Step 2:** Identify the fragmentation pattern. Next to the molecular bar is the base peak observed at 241 of m/z value, followed by some peaks at 235, 207, 165, and so forth. For some reasons, the fragmentation pattern is relatively similar to diazepam. Not only their structures are so close, which their difference is only due to one methyl branch, but also their fragmentation patterns are also similar. Thus, several assumptions are reliable as well.
- 3) **Step 3:** Determine for significant ion series. As the base peak is detected at 241 m/z value, we can easily refer the lose mass as typical for ketones series (m/z is 29). The only -Cl in the structure is fragmented resulting mass reduction from 284 to 249 (-35 m/z). Or the alternative is ketones first, and followed by -Cl as detected at peak of 207 m/z value. Similar to diazepam pattern.
- 4) **Step 4:** Identify isotope pattern. Since nordazepam has one Cl, its isotope pattern can be simply distinguished as we see in **Figure 7** of Cl isotopes pattern and its relative intensity. The fragment in spectrum of 249 m/z show typical isotope bar for one Cl. Once again, structure assumptions that we use for diazepam are also reliable for nordazepam. It reveals the conclusion that our procedure is consistent by results. The similar way seems possible for oxazepam and other benzodiazepine derivatives.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. **Figure 36** shows some most probable cleavages next to the hetero atom exist (i.e., nitrogen). The cleavages pattern is relatively similar with diazepam.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as nordazepam

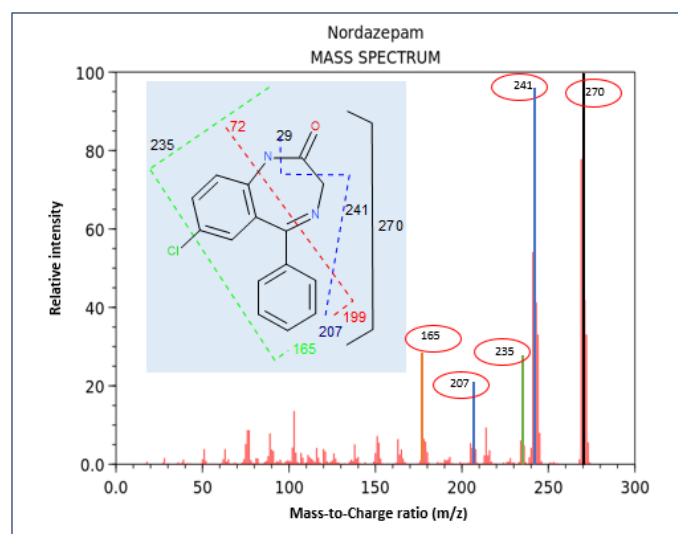


Figure 36. Mass spectrum of Nordazepam and its interpretation result using step-by-step procedure.

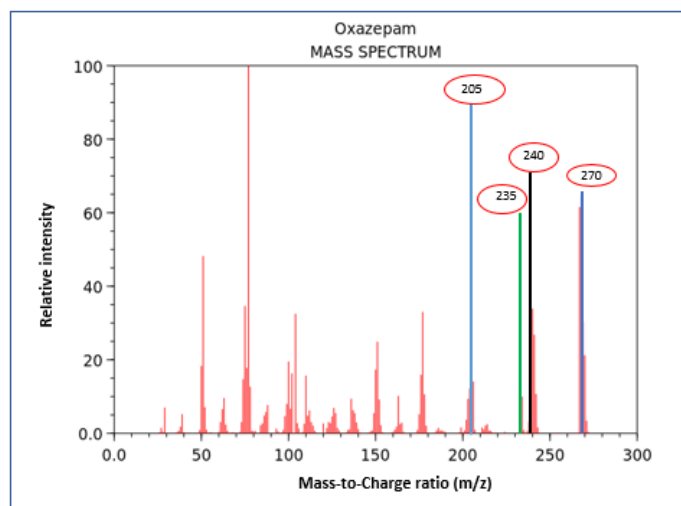


Figure 37. Mass spectrum of oxazepam and its interpretation result using step-by-step procedure.

3.4.3 Sample 3: Oxazepam

Our third sample of very complex compound is oxazepam. It is also benzodiazepine derivate, as we have for diazepam and nordazepam, previously. Based on chromatogram in **Figure 34**, we know that oxazepam is retained at 6.29 min, right after nordazepam detection. Hence, to identify the compound, we proceed its individual mass spectrum using step-by-step procedure:

- 1) **Step 1:** Identify the molecular ion. Oxazepam has relative molecular mass around 286.7. Unfortunately, first appeared peak on the spectrum as shown in **Figure 37** is 268.7 m/z value. The phenomenon is similar to MTBE as what we found priorly. Hence, even though the spectrum has no its molecular detected as we expected, but from the structure and the molecular weight gap, we can justify its possibility.
- 2) **Step 2:** Identify the fragmentation pattern. First peak belongs to the compound base peak observed at 270 of m/z value, followed by some peaks at 240, 235, 205, and so forth. First fragment loss belongs to hydroxyl group (m/z=17). It is straightly removed when the ionization process conducted, and the structure remaining is nordazepam (literally). Followed by ketones removal, which seen from m/z 240 (-30) as what we saw earlier in diazepam and nordazepam. 235 m/z value reveals after -Cl leaves the structure. While m/z at 205 represents the -Cl group removal following the ketones group. Next fragmentation patterns are identic to nordazepam group with few relatively shifting in m/z peak values.
- 3) **Step 3:** Determine for significant ion series. As the base peak is detected at 270 m/z value, it refers the lose mass as typical for hydroxyl group series (m/z is 17). Followed by mass losses around 30 from ketones group series.
- 4) **Step 4:** Identify isotope pattern. Based on **Figure 37**, isotope pattern is most observed at m/z peak 235 which indicative for one Cl.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. The pattern is mostly similar with nordazepam. So, the cleavages series also follow nordazepam. **Figure 38** shows the interpretation result using step-by-step procedure in details.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as oxazepam. **Figure 38** represents the interpretation result.

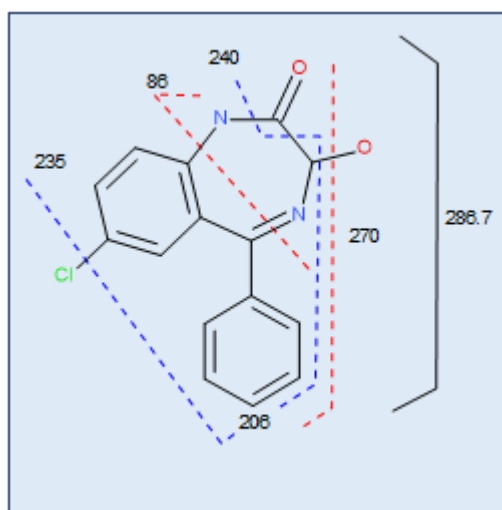


Figure 38. Interpretation result of oxazepam mass spectrum using step-by-step procedure.

3.4.4 Sample 4: Thiabendazole

We have thiabendazole (**Figure 40**) as the fourth sample of highly complex compound for GC/MS spectra reading and interpretation using step-by-step procedure. Thiabendazole is a derivate of benzimidazole. In this case, the analysis for thiabendazole is chosen from grapefruit extract mixture ([Schachterle, 2010](#)). **Figure 39** shows its chromatogram using GC/MS analysis.

We conduct spectrum interpretation using step-by-step procedure as follow:

- 1) **Step 1:** Identify the peaks display. **Figure 39** depicts multiple peaks detected of grapefruit extract analytes. Some strong peaks are distinguished as retained components separation are proceeded.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. Based on the report ([Schachterle, 2010](#)), the chromatogram has been compared to the standard resulting the identification of thiabendazole chromatogram peak at RI 15.77 min.

Mass spectrum for individual compound interpretation is conducted to identify the molecules. Following are the step-by-step procedure:

- 1) **Step 1:** Identify the molecular ion. Thiabendazole has relative molecular mass 201. **Figure 40** shows that the molecular ion peak is identic to thiabendazole.
- 2) **Step 2:** Identify the fragmentation pattern. First peak belongs to the compound base peak observed at 174 of m/z value, followed by some peaks at 129, 89, 84, and so forth. First fragment loss belongs to $-CN$ group ($m/z=27$). Followed by amine-based compounds fragmented from benzyl-imine, which seen from m/z 91. The fragmentation also can be drawn by benzene ring removal which is detected from fragments at m/z values 72 and 129.
- 3) **Step 3:** Determine for significant ion series. The significant ion indicatives only shown by benzene which reveals at m/z 70's.
- 4) **Step 4:** Identify isotope pattern. Based on **Figure 40**, isotope patterns of S atom are observed at m/z peak 203 and 131 out of noted peaks. These peaks are indicatives as seen in **Figure 11**.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. Four cleavages pattern are simulated close to the hetero atoms, and resulting significant fragments for identification of the compounds as seen in **Figure 40**.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as Thiabendazole. **Figure 40** illustrates the interpretation result using step-by-step procedure.

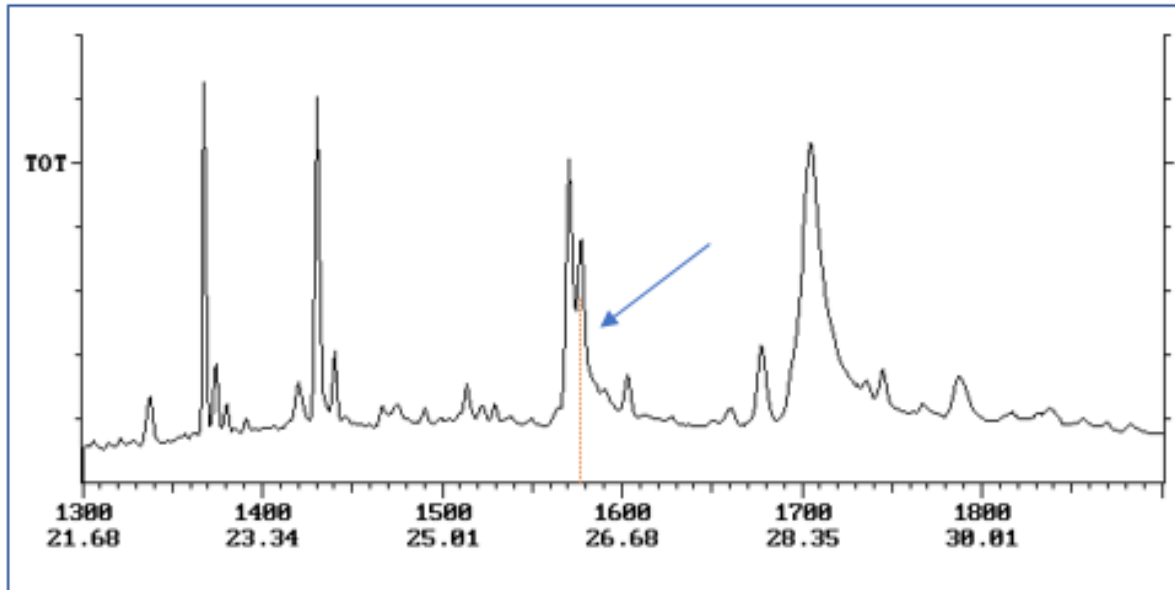


Figure 39. GC/MS chromatogram of grapefruit extract (Schachterle, 2010).

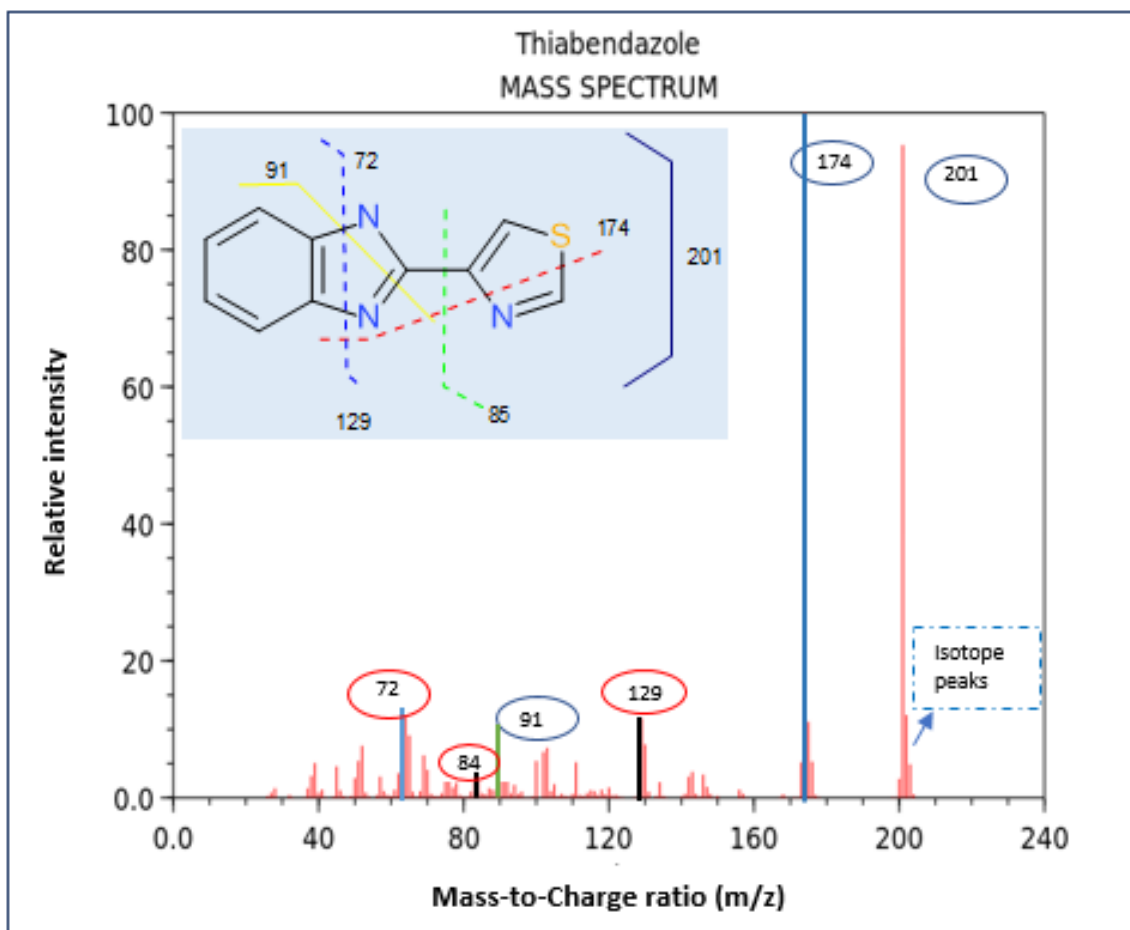


Figure 40. Thiabendazole mass spectrum and its interpretation result using step-by-step procedure.

3.4.5 Sample 5: Testosterone

Our last sample is testosterone. The structure is relatively complex, as shown in **Figure 41**. [Fitzgerald et al., \(2010\)](#) reported analysis of testosterone using GC/MS analytical method from human serum. The GC/MS total ion chromatogram can be seen in **Figure 42**.

Based on the chromatogram in **Figure 42**, the interpretation steps are following:

- 1) **Step 1:** Identify the peaks display. **Figure 42** shows multiple peaks detected with 2 of them predominates the abundances. The separation tends to be well proceeded as the retention time are clear for the separated components.
- 2) **Step 2:** Identify the order of the peaks along the RI axis. Two of the most massive compounds are retained at 7.40 and 7.56 min, respectively. They are the epimers of testosterone pentafluorobenzyl oxime (after derivation by protecting group).

Mass spectrum interpretation used here is based on NIST spectral data for the purpose of simplification. Following are the step-by-step procedure:

- 1) **Step 1:** Identify the molecular ion. Testosterone (underived) has relative molecular mass 288. **Figure 43** shows that the molecular ion peak is identical to testosterone.
- 2) **Step 2:** Identify the fragmentation pattern. First base peak appears at 246 of m/z value (-42), followed by some significant peaks at 165, 147, 123, 79, and so forth. First fragment loss belongs to $-CH_3$ group ($m/z=15$), resulting peak at m/z 273 but its intensity is relatively low. Fragmentation splits to both big molecules is observed at m/z 165, which the fragment resulted also observed at m/z 123. The fragmentation illustration is shown at **Figure 43**.
- 3) **Step 3:** Determine for significant ion series. The significant ion indicatives are observed from methyl at m/z 273 and 147. Ketones group series also identified at m/z 246 (-42).
- 4) **Step 4:** Identify isotope pattern. In this case, there is no S, Cl, Br atoms. The isotopes of C are also unidentified significantly to the mass fragments.
- 5) **Step 5:** Examine the more significant fragments and consider possible hetero atom induced cleavages. There is no hetero atom expected in the main structure.

Based on the interpretation of mass spectrum using step-by-step procedures, we can conclude that the compound is identified as testosterone. **Figure 43** illustrates the interpretation result using step-by-step procedure.

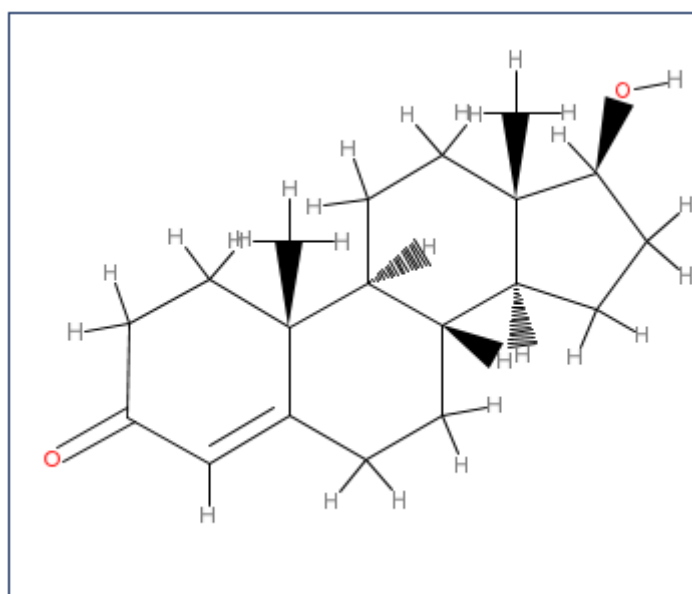


Figure 41. Testosterone structure.

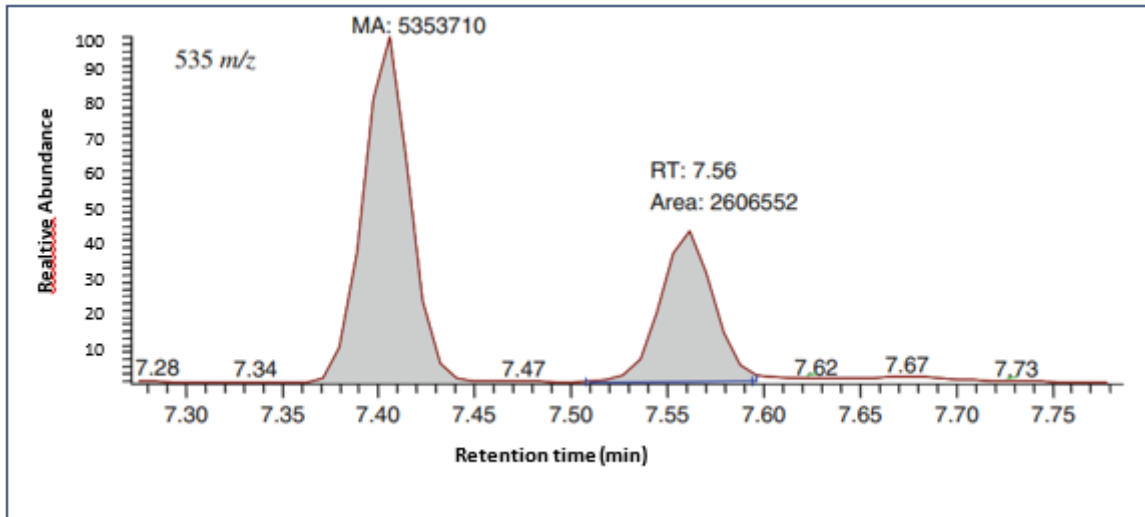


Figure 42. GC/MS chromatogram of Testosterone (Fitzgerald *et al.*, 2010).

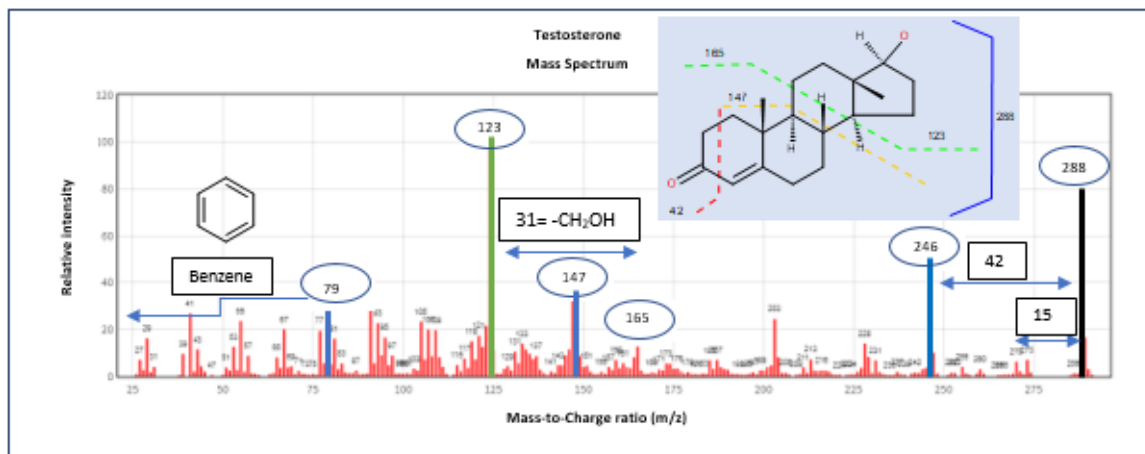


Figure 43. Testosterone mass spectrum with its ab initio interpretation using procedural steps.

4. CONCLUSION

Gas chromatography / mass spectrometry (GC/MS) is the most ubiquitous analytical method for the identification of mostly organic substances in complex matrices. This study shows the simplest way to understand the result of GC/MS analysis. Comparative study using chemical drawing software also conducted to achieve better understanding of the fragmentation mechanism and mass spectra reading and interpretation. The result shows that simulated fragmentation is consistent with the experimental one. Hence, we believe that this article can be adopted in a practical basis for understanding and interpreting the GC/MS data output.

5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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