# Introducing Students to Gas Chromatography−Mass Spectrometry Analysis and Determination of Kerosene Components in a Complex Mixture

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S *Supporting Information*
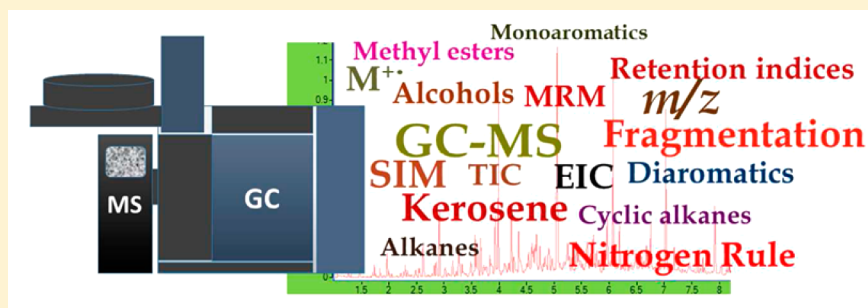
ABSTRACT: Gas chromatography−mass spectrometry (GC−MS) and GC−tandem MS (GC−MS/MS) are useful in many separation and characterization procedures. GC−MS is now a common tool in industry and research, and increasingly, GC−MS/MS is applied to the measurement of trace components in complex mixtures. This report describes an upper-level undergraduate experiment to understand aspects of GC−MS analysis for a mixture of compounds, carry out a protocol for selected ion monitoring, and then determine a number of kerosene component classes using GC−MS. The first exercise analyzes a standard solution containing different compounds by using GC−MS, obtains characteristic ions, conducts library searching, calculates retention indices, and displays various extracted ion plots. Selected ion monitoring is then conducted based on retention time windows and characteristic ions for this sample. Kerosene is then analyzed by GC−MS to identify various classes of compounds using extracted ion plots to support compound class monitoring. The methods described in this report may be readily adapted to other kerosene components and other samples such as pesticides, essential oils, or other samples of interest.

KEYWORDS: *Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Gas Chromatography, Mass Spectrometry*

■ INTRODUCTION

Laboratory activities are integral to the science curriculum: they provide students a learning environment where they develop skills in critical-thinking, communication, and teamwork.[1] Students explore the activities of a scientist, and the experience gives them an opportunity to work with various types of laboratory equipment and learn different techniques.

One technique that will be taught to upper-level undergraduate students is gas chromatography−mass spectrometry (GC−MS). GC−MS merges the capabilities of two analytical procedures: GC uses an inert gas phase to separate different components in a mixture based on their partitioning between gas and stationary phases in a controlled temperature oven, while mass spectrometry measures mass-to-charge ratios (m/z) and abundances of gas-phase ions at high vacuum, arising from ionization and fragmentation of the molecule in a well-defined manner. The reproducibility of fragmentation allows database searching of spectra to match with the analyzed molecule. Combining these two tools allows separation and identification of each component in a mixture.[2]

With increasing availability of GC−MS instruments, this *Journal* has reported several laboratory experiments that incorporate GC−MS in the laboratory. For example, GC−MS can identify and quantify components of coffee samples,[3] water contaminants,[4,5] scent components in perfumes,[6,7] and volatile components in plants[8] and fuels.[9,10] In some schools, GC−MS is part of the official undergraduate laboratory curriculum.[11−14] This shows that the technique is widely accepted as integral to the students' learning experience.

This paper describes the development of an upper-level undergraduate laboratory activity on an application of GC−MS to two mixtures. Students are progressively guided through the

analysis of a standard mixture containing analytes from different chemical classes, obtaining the total ion chromatogram (TIC) and other relevant data. Extracted ion chromatogram (EIC) is introduced to obtain plots of specific ions to display compounds yielding the target ion. Selected ion monitoring (SIM) analysis provides experience with a protocol to select retention windows and target ions to confirm compound presence. Finally, an exercise that challenges identification of different chemical classes that are present in kerosene is completed. Additional information is provided for an extension study using MS/MS techniques such as product ion scans and multiple reaction monitoring (refer to the Supporting Information).

This exercise has been conducted by senior analytical chemistry class students for about 10 years. With about 25 pairs in a year, around 250 iterations of the exercise have been conducted. Other University chemistry departments have also adapted the experiment in their own classes. Conducting real-time GC−MS analyses with rapid online library searching of mass spectra mimics the workflow in a routine analytical lab; this aspect of the laboratory is always appreciated by students. EIC is often met with surprise at its ability to selectively target specific compounds in a complex matrix. This exercise reinforces lectures on critically assessing library searches, the importance of good peak separation, and the use of retention indices for qualitative analysis.

■ LEARNING OUTCOMES

At the end of the activity, the students are able to

(1) Explain the operation of GC−MS.
(2) Consider library searching and limitations.
(3) Perform simple interpretation of a mass spectrum, noting differences between compounds with widely varying molecular ion abundance.
(4) Perform extracted ion analysis by identifying specific chemical classes based on characteristic ions.
(5) Conduct selected ion monitoring.
(6) Identify chemical classes and specific compounds present in kerosene.

■ MATERIALS AND METHODS

Materials

A series of alkane standards (C8−C12) were obtained from Sigma-Aldrich. GC-grade hexane and analytical-grade dichloromethane were obtained from Merck Chemical Co (Germany). Chemicals for standard preparation were obtained from various sources according to availability in our Chemistry laboratory. Kerosene was obtained from a convenience shop.

Preparation of Standard Mixture

The following mixture is provided as a prepared "unknown"; additional compounds may be added as required according to the interests of the laboratory coordinator.

A 25 mL volumetric flask was triple-washed with dichloromethane. The following were then added: 5 mL of dichloromethane, 0.1 g of benzoic acid, 0.1 g of naphthalene, and 0.5 mL of each of chlorobenzene, bromobenzene, 1,3-dichlorobenzene, hexane, heptane, octane, decane, dodecane, o-xylene, p-xylene, 1-pentanol, nitrobenzene, and cyclohexane. The flask was filled to the mark with dichloromethane and mixed well; this was mixture A.

To a 5 mL volumetric flask that was triple-washed with dichloromethane, the following were added: 2 mL of dichloromethane, 0.5 mL of mixture A, 0.8 mL of dodecanoic acid methyl ester (100 ppm), 0.3 mL of benzoic acid methyl ester, and 0.5 mL of caffeine (1000 ppm). The flask was filled to the mark with dichloromethane and mixed well.

CARE: Dispose of all solutions of chlorinated solvent in a designated waste container.

Preparation of Kerosene Sample

A 100-fold dilution of kerosene with hexane was prepared.

GC−MS Analysis

GC−MS analysis may be conducted on any suitable instrument. For this report, Agilent 7000 triple quadrupole GC−MS system was used: mass selection was conducted via one of the quadrupoles. The experiment has also been conducted on an older Agilent 5890 GC−5970 MSD, on a new model Bruker Scion 456 GC TQ MS, and a new model PerkinElmer Clarus SQ 8T MS with a Clarus 680 GC.

One microliter of sample was injected into the split/splitless injector. Our laboratory class also studies operation of the split/splitless injector following the procedure outlined in an article previously published in this Journal.[15] The instrument was controlled using MassHunter Workstation Software Version B.06.00; to identify each peak, NIST MS library 2011 was used. The run parameters were as follows: inlet and detector temperatures of 250 °C; injection split ratio at 50:1 (reduced to 20:1 after 2 min); DB-5 ms column (30 m × 0.25 mm I.D. × 0.25 μm d_f; Agilent Technologies); constant carrier gas (He) flow rate of 1.5 mL/min; oven temperature program of 50 °C (1 min) to 300 °C (1 min) at 15 °C/min; solvent delay of 1 min; total ion chromatogram (TIC) acquisition mass range of m/z 40−400 u (u: unified mass unit).

■ HAZARDS

Appropriate protective clothing as prescribed by personal protective equipment (PPE) guidelines must be worn by students in the laboratory. Many organic solvents are flammable and must be kept away from spark sources and open flames; detailed information is available on each chemical's safety data sheet (SDS). Mixtures must be prepared in a fume hood to avoid irritation when inhaled. When handling electrical equipment, including computer-controlled instruments, the risk for electric shock to occur may be minimized by keeping liquids away from the work bench. All chemicals must be disposed of in the correct waste containers.

■ RESULTS

The first analysis was on the standard mixture. Figure 1 shows its TIC result, while Table 1 shows the data obtained for each peak.

A sample pro forma sheet for students is provided in the Supporting Information. The retention times, names, and library match scores were obtained from the NIST library search, and the diagnostic ions were chosen based on their abundance in the MS spectra. The retention indices, I, may be expressed with relation to straight-chain alkanes using the following equation for temperature-programmed methods:[10]

$$I = 100\left(n + i\frac{t_r(\text{unknown}) - t_r(n)}{t_r(n + i) - t_r(n)}\right)$$
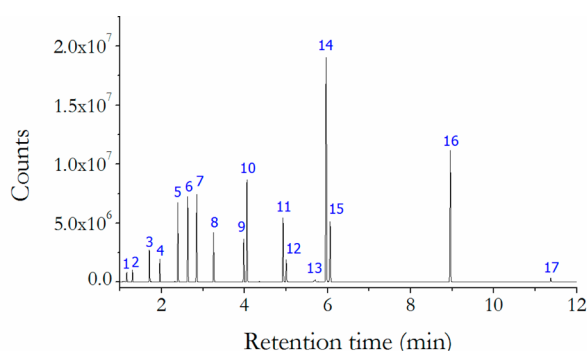
Figure 1. TIC for the standard mixture. Numbers correspond to analytes in Table 1.

In this equation, n is the number of carbons in the smaller alkane, $n + i$ is that for the larger alkane (in the case of successive alkanes, $i = 1$), and t_r is the compound's retention time. For compounds that are not bracketed between alkanes, the retention index is undetermined. I data may be compared with those in the NIST library, if available.

To search for target ions in specific time windows, the standard mixture may be analyzed using selected ion monitoring (SIM). Table 2 shows an example of time windows and ions to monitor within each. The ions may be chosen based on the diagnostic ions identified in the total ion scan or any ions that the students wish or choose to monitor.

The chromatogram from the SIM run is shown in Figure 2.

The TIC of the diluted kerosene sample is shown in Figure 3.

To determine if specific chemical classes are present in kerosene, extracted ion chromatograms (EICs) may be studied. As an example, the EICs of m/z 91 (common daughter ion for monoaromatics) and m/z 128 (to indicate naphthalene and also nonane) are shown in Figures 4 and 5.

■ DISCUSSION

GC−MS Analysis of Mixture

In this 3-h laboratory exercise, students are guided through a progressive analysis of a standard mixture, which represents different chemical classes: alkanes, from which retention indices

Table 2. Example SIM Strategy

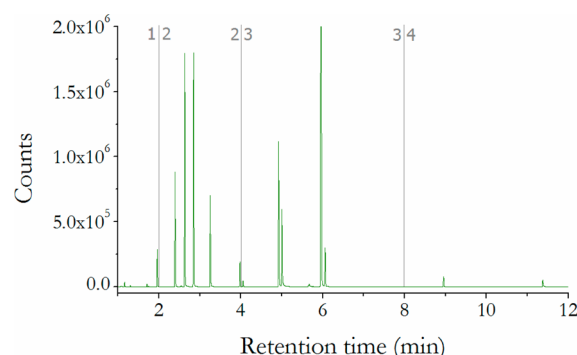| SIM Window	Time Window Start Time	Chosen (min) End Time	Target Compounds (Based on Peak No. in Table1)	Ions Chosen
1	1.0	2.0	1, 2, 3, 4	45, 83, 85
2	2.0	4.0	5, 6, 7, 8, 9	77, 85, 91
3	4.0	8.0	10, 11, 12, 13, 14, 15	77, 85, 105, 128
4	8.0	12.0	16, 17	85, 194, 214

Figure 2. SIM chromatogram for standard mixture according to the protocol in Table 2. The vertical gray lines with numerals represent separate zones (SIM windows) of selected chosen ions, corresponding to the windows defined in Table 2.

may be calculated and which give strong class-type fragmentation (yielding common m/z values of 43, 57, 71, 85 ions) but low $M^{+\bullet}$ ion abundance; aromatics, with strong $M^{+\bullet}$ ion intensities and often little fragmentation; chlorinated, dichloro-, and bromo-aromatics, which show diagnostic isotope ions in the molecular ion region, and loss of the halogen which alters the isotope ratio; esters, which show characteristic fragmentations; an alcohol, which has essentially no $M^{+\bullet}$ ion; a selection of xylenes, the o- and p-isomers of which can be resolved, but no definite library match can be assigned; methyl benzoate and benzoic acid, which show the "overload" poor

Table 1. Peak Data for the Prepared Sample Mixture

| Peak No.	t_R (min)	Name	Retention Index	Library Match Score	Diagnostic Ions
1	1.164	Cyclohexane	undetermined	93.8	56, 84, 41
2	1.301	Heptane	700	96.2	43, 57, 71
3	1.707	1-Pentanol	762	89.5	42, 55, 70
4	1.959	Octane	800	96.2	43, 71, 85
5	2.393	Chlorobenzene	843	97.9	112, 77, 114
6	2.631	p-Xylene	866	98.1	91, 106, 77
7	2.846	o-Xylene	888	97.6	91, 106, 77
8	3.254	Bromobenzene	928	95.7	77, 156, 51
9	3.981	Decane	1000	96.3	43, 57, 71
10	4.057	1,3-Dichlorobenzene	1007	95.7	146, 111, 75
11	4.926	Nitrobenzene	1091	96.4	123, 77, 51
12	5.001	Methyl ester benzoic acid	1098	98.5	105, 136, 77
13	5.702	Benzoic acid	1166	96.7	122, 105, 77
14	5.964	Naphthalene	1191	99.1	128, 102, 64
15	6.060	Dodecane	1200	97.6	57, 71, 85
16	8.953	Methyl ester dodecanoic acid	undetermined	95.6	74, 87, 214
17	11.371	Caffeine	undetermined	91.9	194, 109, 55
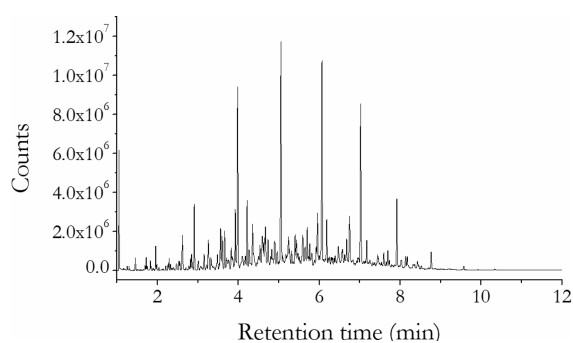
Figure 3. TIC for kerosene.
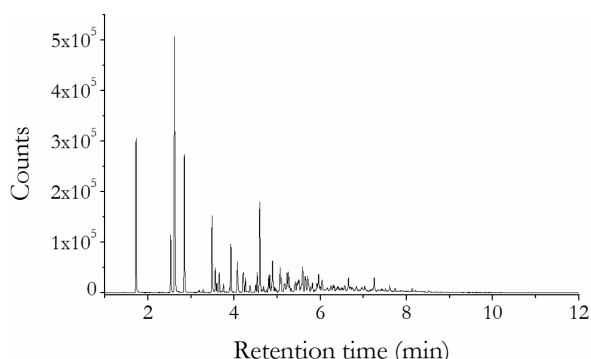
Figure 4. EIC for m/z 91, showing monoaromatics such as toluene (t_R = 1.72 min) and xylenes (t_R = 2.6−2.9 min).
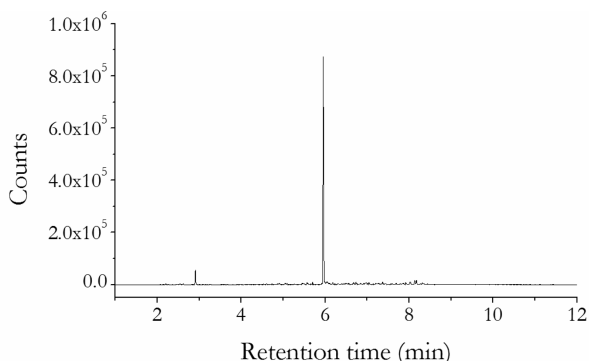

Figure 5. EIC for m/z 128, showing naphthalene (t_R = 5.96 min) and nonane (t_R = 2.91 min). Note that many compounds have a small response for m/z 128 as shown by the baseline perturbations.

peak shape of the acid, and the differences between spectra regarding the $M^{+\bullet}$ ion intensity but a common m/z 105 ion of the benzyl ion; a solute with a N atom, to illustrate the nitrogen rule; and the effect of a cyclic alkane structure, which gives an m/z 83 peak instead of m/z 85.

The student reports major ion abundances and masses, retention times, molar mass, and NIST library records. Retention indices (I) can be calculated and the value of searching based on both MS and I values proposed; some compounds may have I values in the NIST library based on the 5% phenyl phase. In many cases, there are multiple entries of the same compounds according to the original data source. Interpretation of a few of the spectra can be undertaken.

EIC on the standard mixture allows the students to selectively print out ions of interest. Thus, selection of mass m/z 57, 71, or 85 will predominantly display the alkanes or any other compound with these ions. Mass m/z 83 will display the

cyclic compound, which has 2 u lower mass fragment than the alkane due to its cyclic structure. Mass m/z 91 will display monoaromatics, while mass m/z 128 selects naphthalene, and so forth. This will be useful for the kerosene part of the exercise later. Supporting Information Part B reports a selection of other EIC plots.

SIM Analysis of Mixture

The above results are then used to develop a SIM strategy that only monitors for selected ions in defined time windows. If a compound does not have any of the SIM ions in a given window, it will simply not be measured; no peak will be recorded. The role of quantification and qualification ions in the SIM analysis can be highlighted. The qualification ion should confirm the presence of the compound and is especially useful in a MS/MS analysis.

Analysis of Kerosene

Finally, a kerosene sample is analyzed. Because of time constraints, interpretation of chemical classes is based on EIC plots. The student is asked to identify "five different classes" of compounds. We have to be a little open to what constitutes a chemical class. We have "alkanes", but within this, straight chain, branched, and cyclic alkanes. Within "aromatics", we have mono-, di-, and triaromatics. Olefins might also be checked. If we wish to try a diesel sample, and choose a sample with "biodiesel" components (e.g., with some fatty acids content blended with regular diesel), we will also find fatty acid methyl esters. Some biofuels may have ethanol as the "bio" ingredient.

The student is directed to select various peaks, a random process works acceptably, and deduce what they might be. Although this "random process" is informative, it can be frustrating if not supported by some informed decision-making. Fortunately, part 1 has already identified retention times of alkanes, which are among the largest peaks in kerosene, so hopefully the astute student might recognize this. After locating the peaks of alkanes (m/z 85) at their respective t_R, the student is then encouraged to think about other components of kerosene. The prospect of aromatics suggests an EIC plot of m/z 91 (monoaromatics). The student might also propose testing EIC m/z 83 (cyclic compounds). Then, by directing attention to the later part of the kerosene trace, they might strike the diaromatics. One further "hint" is to ask, "If the sample includes a single ring aromatic compound, what other type of organic compound could it contain?" The answers to this question are surprisingly broad! However, eventually, they might suggest two-ring compounds, and to locate these? An EIC plot for m/z 128 can be displayed, showing naphthalene (very strong $M^{+\bullet}$ ion) and nonane (very low abundance $M^{+\bullet}$ ion). Once they congratulate themselves for this insight, you can ask, "if you have found naphthalene, what other compounds should you now look for?" Two answers: a three-ring compound, or the methylated analogues. Both can now be tested using EIC protocols. The methyl naphthalene (m/z 142) should be obvious since if kerosene has methylbenzene and xylene, then methyl naphthalene (and di- and trimethyl) is a logical guess. The number of isomers of these mono-, di-, and triaromatics is interesting to discuss. Additional EIC plots are provided in the Supporting Information, Part C.

Preparing the report for this exercise might be long, but it should ultimately be very instructive and informative. It can also provide useful questions and case studies in lecture-style material and tutorials depending on the initiative of the

instructor. For instance, this approach is also used for pesticides analysis and widely for essential oils, especially with retention indices. However, the most interesting is the role of drug testing and GC and HPLC−MS methods in doping control, and the use of MS/MS for these compounds.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00830.

>Suggested student handouts; additional results; information on a possible extension study (PDF, DOC)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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