

CHAPTER 8

Sample Laboratory Experiments

8.a Analytical Experiments without an External Reference Standard;
Conformational Identification without Quantification.

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CAUTION: Do not repeat this experiment since you will plate out sugars on your injection system and permanently contaminate it.

One of the most powerful applications of an MS system is its ability to identify an analyte without a reference compound. In GC, reference compounds are needed to determine the retention time, the criteria for identification in GC. In MS, the spectrum is the identifier since it can be compared to thousands of reference spectra and a unique match is normally achieved. The laboratory exercises below illustrate the power of MS in identifying unknown analytes in a variety of samples.

Identification of Components in Liquors and Distilled Spirits

Distilled Spirits contain a range of flavors that can be identified by GC-MS. Not only can an analyst tell what type of liquor is present (i.e. gin versus whiskey) but they can also compare the presence and abundance of select flavor compounds between different brands of a given type of liquor.

NOTE/WARNING: Some liquors contain nonvolatile components that will coat out on the glass liner in the injector port. The injector liner may need to be cleaned or replaced after completing this laboratory exercise to avoid damage to the GC column.

Experimental Procedures

Chemicals and Supplies:

Pure samples of a variety of liquors. One approach is to contrast types of liquors (rum versus gin versus whiskey). Another approach is to contrast brands (spiced liquor versus pure liquor).

GC-MS Settings:

Capillary Column: DB-5
Poly(phenylmethyl dimethyl) siloxane (5
% phenyl)
30 m x 0.25 mm; 0.25 μ m phase coating

Injection Volume: 1.00 μ L
Splitless Injection for: 0.50 min.
Split Flow Rate: 50 mL/min.
Column Flow: 1.3 mL/min.
Linear Velocity: 42 cm/s
Injector Temperature: 250°C

MS Transfer Line

Detector Temperature: 230°C

Quadrupole Temperature: 150 °C

Oven Program: Initial Temp. at 60.0°C for zero minutes, 2.0°C
to 150°C, hold for zero minutes. Post Temp. at 280°C for 10 minutes.

Total Run Time: 45 min.

Procedures:

Analyze a variety of liquor samples on a GC-MS using the instrumental conditions given above.

RESULTS:

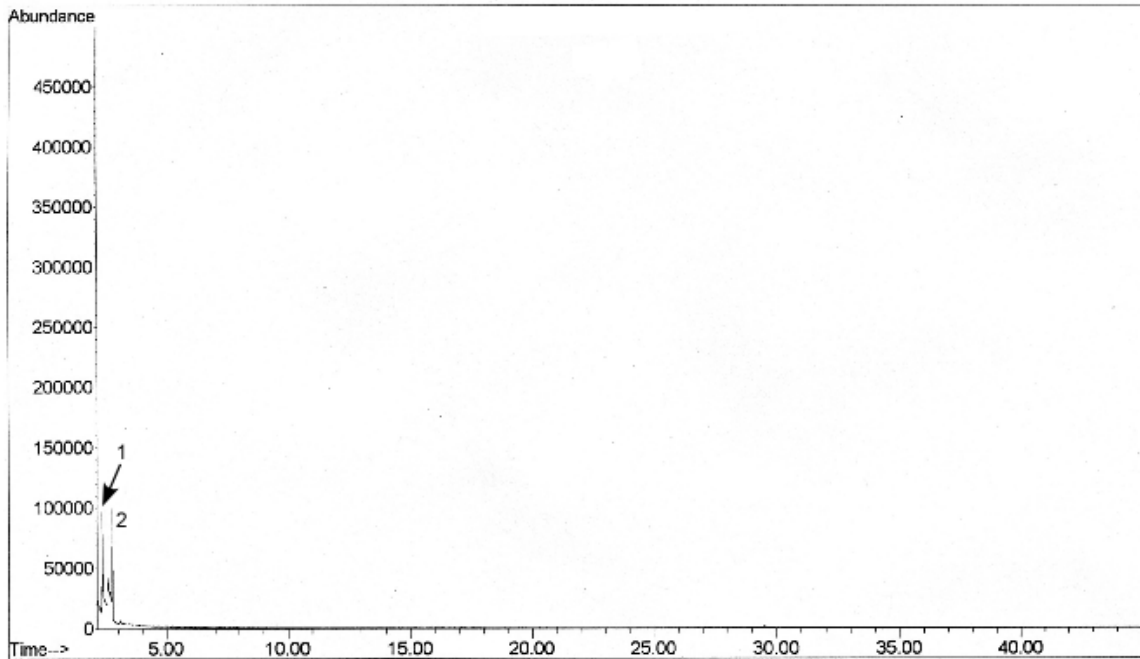
It is relatively easy to distinguish between most types of liquors. However, pure liquors (unspiced) such as rum and vodka produce similar chromatograms and only contain ethanol, water, and a few trace longer chain alcohols. Other liquors, as shown below, are easily distinguished.

All of the compounds identified in the chromatogram below were conclusively identified by the spectral library (typically 99 percent confidence/probability) and are known to be present in the liquors based on a scientific literature search or from common information found on company web sites or web searches. An interesting project is to Goggle some of the compounds identified in

the spectra and research their origin and why they are added to a specific liquor.

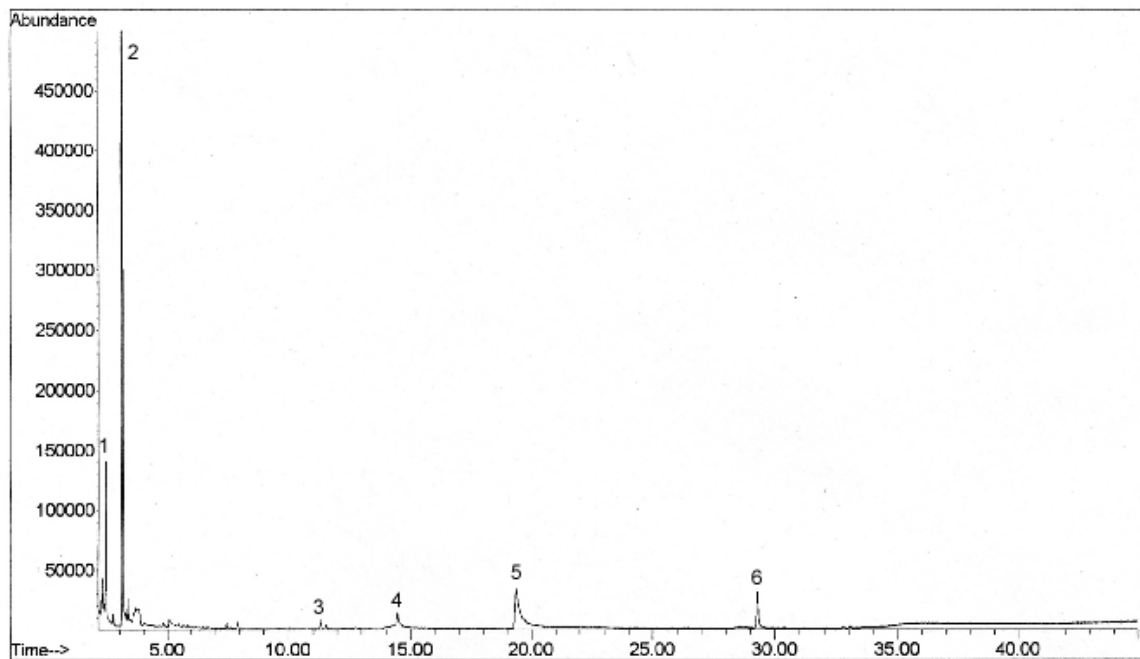
Rum: Rum is a fermented beverage made from sugarcane byproducts such as molasses and juice. After fermentation, it is distilled as a clear liquid. Double distillation yields the light rums, while single distillation will yield darker rums that were originally thought of as being of lower quantity. From here the process becomes brand specific and the initial rum can be aged in a variety of barrels, including oak to impart strong flavors, or filtered through charcoal to remove colors. Spices or color agents are then added.

Figures a-1, 8a-2, and 8a-3 are chromatograms for Bacardi Gold, Captain Morgan, and Citrus Rum, respectively. Note the lack of compounds in the relatively pure Bacardi Gold rum, only 3-methyl-1-butanol and acetic acid are present in measurable quantities. Bacardi Gold has little presence of the oak flavor compounds such as those found in the other two rum beverages. Captain Morgan's flavor is characterized by additional compounds, most notably oak flavors and vanilla. Citrus Rum, a specifically spiced rum, not surprisingly contains almond, orange, cocoa, fruit, and lemon flavors, as well as extracts from the oak barrel aging process.



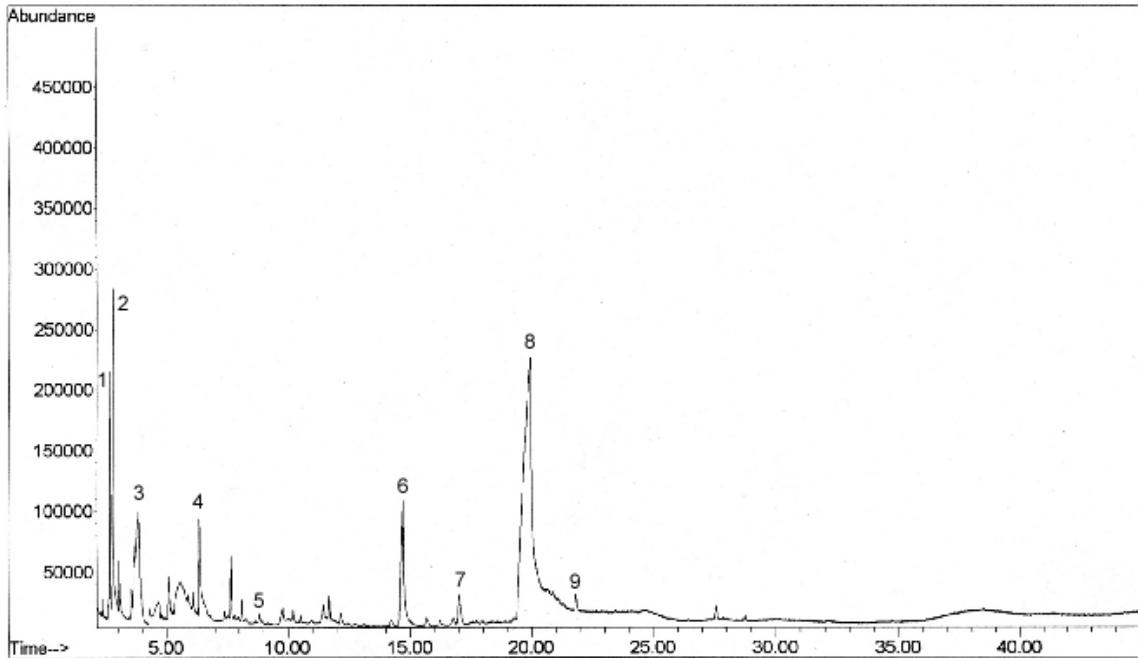
- 1) 3-methyl-1-butanol
- 2) acetic acid

Figure 8a-1. Chromatogram of Barcardi Gold.



- 1) acetic acid
- 2) 1,2-dihydroxypropane
- 3) 5-methylbenzene-1,3-diol
- 4) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (toasty caramel aroma of heated oak)
- 5) 5-hydroxymethylfurfural (wood flavor)
- 6) 4-hydroxy-3-methoxy-benzaldehyde (vanilla flavor)

Figure 8a-2. Chromatogram of Captain Morgan.



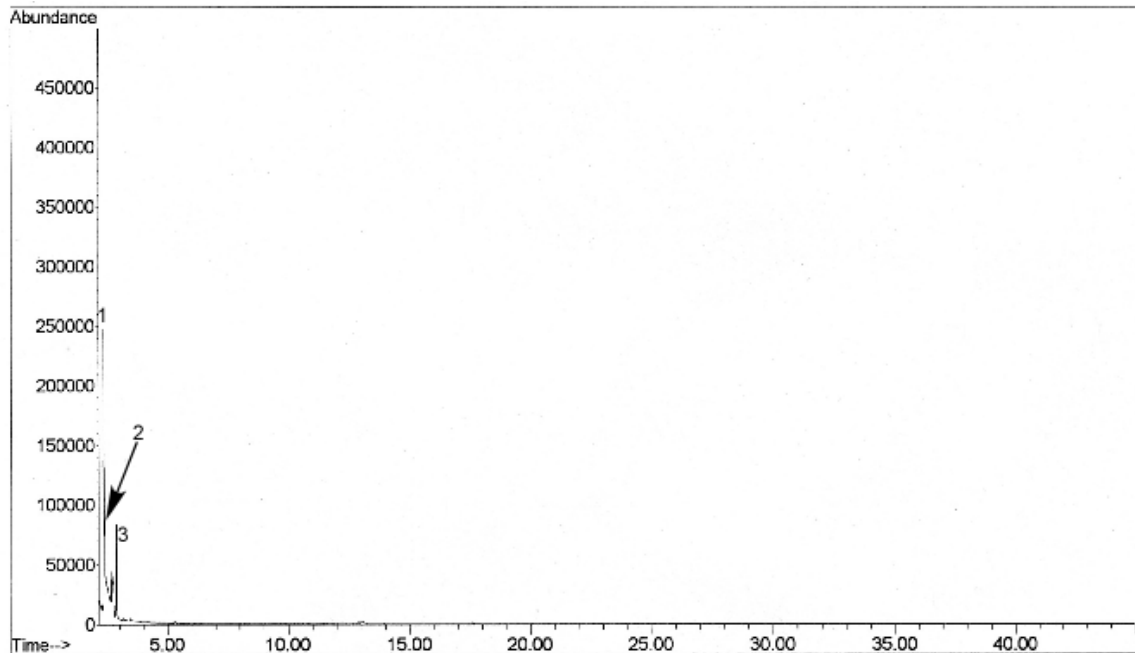
- 1) formic acid
- 2) acetic acid
- 3) 2-furaldehyde (wood flavor)
- 4) benzaldehyde (almond flavor)
- 5) limonene (orange flavor)
- 6) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (cocoa flavor)
- 7) α -terpineol (fruit juice flavor)
- 8) 5-hydroxymethylfurfural (wood flavor)
- 9) citral (lemon flavor)

Figure 8a-3. Chromatogram of Citrum Run.

As a side note, most of the components shown in these figures are in the parts per million range of concentrations.

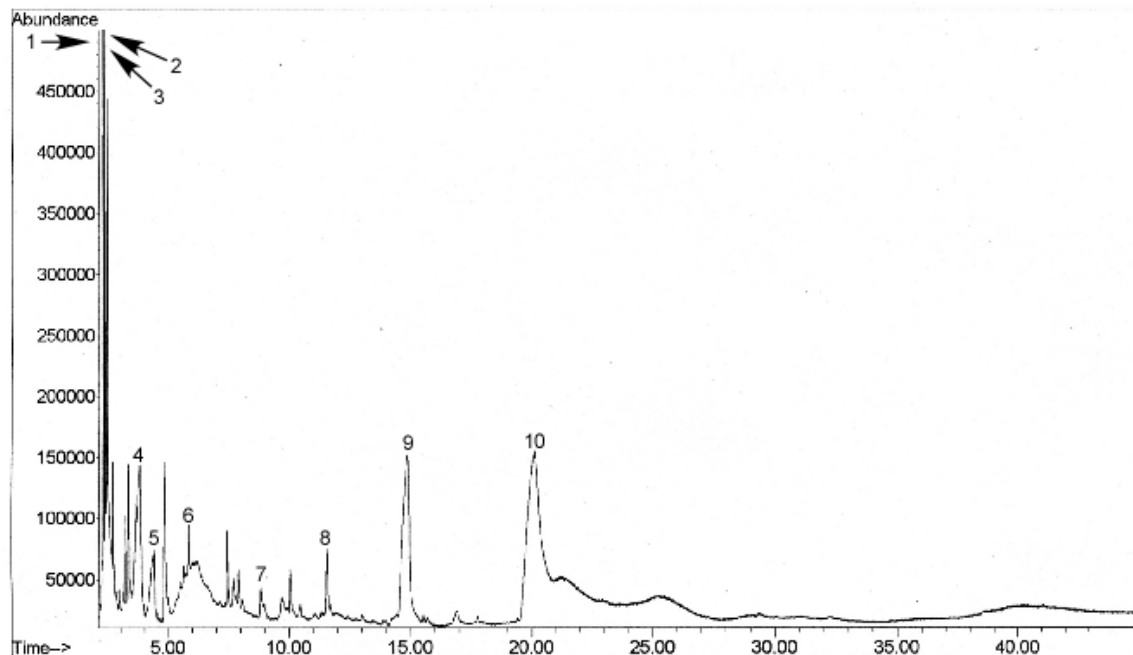
Whiskey: Whiskey (originally Whisky from its origin from Irish monks) refers to a broad range of alcoholic beverages that are distilled from fermented grain mash and aged (matured) in oak barrels (casks). The age of a whiskey refers to its time in the cask (between fermentation and bottling) and the length of aging greatly affects its chemical makeup and taste from the extraction of wood components from the cask. These components include lacone (3-methyl-4-octanolide) that has a coconut aroma, and numerous phenolic compounds. Grains of choice include barley, malted barley, rye, wheat, and corn, and it may be fermented from single or blends

of grains. Published flavoring chemicals include carbonyl compounds, alcohols, carboxylic acids and their esters, nitrogen- and sulphur-containing compounds, tannins and other polyphenolic compounds, terpenes, and oxygen-containing heterocyclic compounds and esters of fatty acids. The nitrogen compounds include pyridines, picolines and pyrazines. After distillation, the flavoring compounds that are common among different brands of whiskey include fusel oils which are higher alcohols that are actually mildly toxic and have a strong disagreeable smell and taste in high concentrations. Hence, these are commonly removed by charcoal and linen filtration. Other common flavor agents in whiskey are acetals, such as acetaldehyde diethyl acetal (1,1-diethoxyethane), the principal flavor agent in sherry. The presence of a buttery aroma is due to diketone diacetyl (2,3-butanedione). Some whiskey blends contain specific flavor agents. Use the chromatograms given below to confirm the presence of these known whiskey components.



- 1) 3-methyl-1-butanol
- 2) 2-methyl-1-butanol (shoulder peak)
- 3) acetic acid

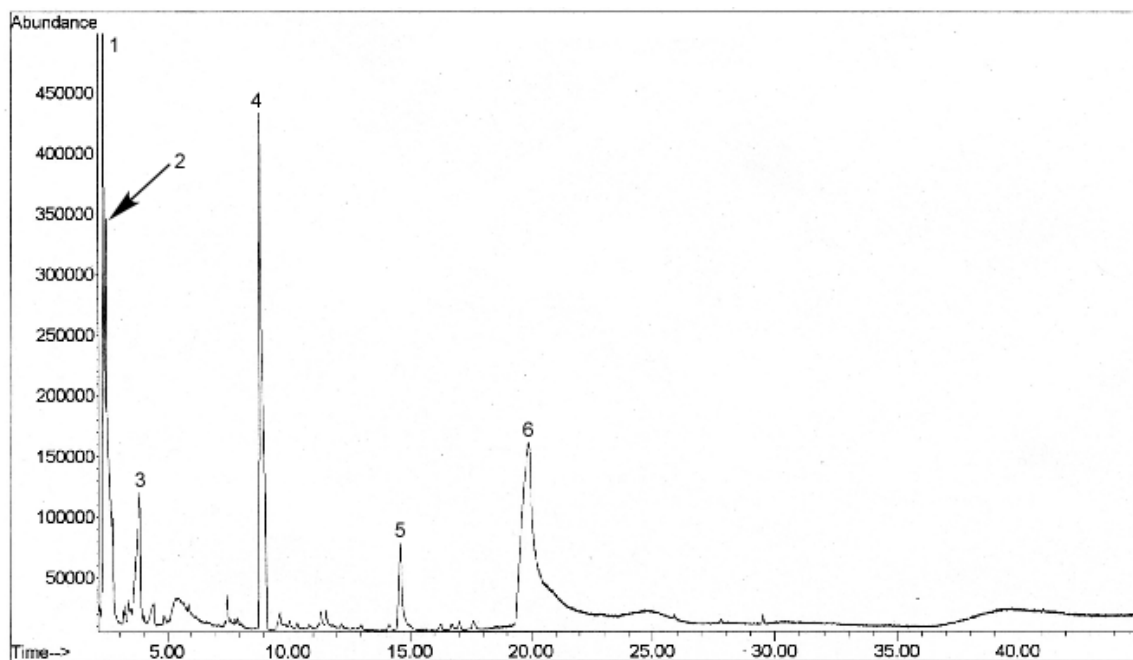
Figure 8a-4. Chromatogram of Crown Royal.



- 1) formic acid
- 2) 3-methyl-1-butanol
- 3) acetic acid
- 4) 2-furaldehyde (wood flavor)
- 5) 2-hydroxymethylfuran (burnt flavor)
- 6) 1,2-cyclopentanedione (wood flavor)
- 7) limonene (orange flavor)
- 8) Methyl 2-furoate (fruity flavor)
- 9) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (cocoa flavor)
- 10) 5-hydroxymethylfurfural (wood flavor)

Figure 8a-5. Chromatogram of Southern Comfort.

Cognac: The cognac used here is Grand Marnier, a blend of cognac. Cognacs are brandies produced from specific white grape varieties. Most cognacs are distilled twice and aged in oak barrels. After the review of the liquors above, the student should be able to predict some of the compounds present in cognac.

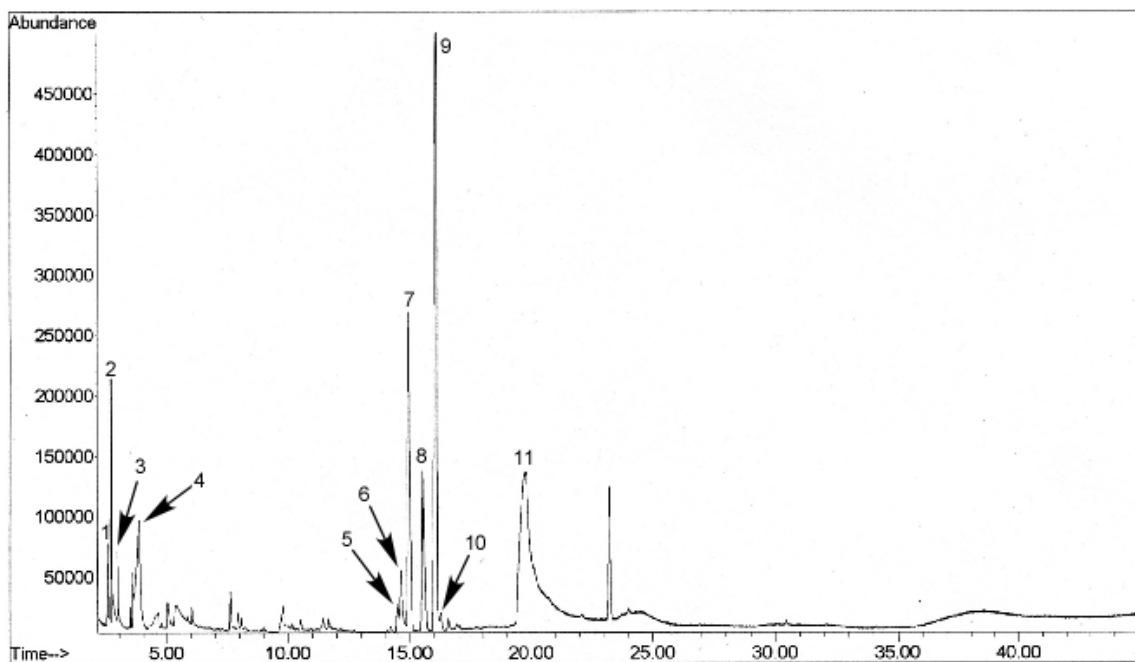


- 1) 1-pentanol
- 2) acetic acid (shoulder peak)
- 3) 2-furaldehyde (wood flavor)
- 4) limonene (orange flavor)
- 5) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (cocoa flavor)
- 6) 5-hydroxymethylfurfural (wood flavor)

Figure 8a-6. Chromatogram of Grand Marnier.

Note that most of the flavor compounds come from the oak barrel or are specifically added.

Peppermint Schnapps: Schnapps is usually a clear, colorless beverage with a light fruit flavor since it is fermented from fruit. The schnapps used in this experiment is infused with peppermint leaf extract or specific flavors (chemicals) found in peppermint. Note the dominant mint flavor compound in the chromatogram.



- 1) formic acid
- 2) acetic acid
- 3) 1-hydroxy-2-propanone
- 4) 2-furaldehyde (wood flavor)
- 5) isopulegol (mint flavor)
- 6) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (wood flavor)
- 7) D-menthone (mint flavor)
- 8) L-menthone (mint flavor)
- 9) 5-methyl-2-(1-methylethyl)-cyclohexanol (mint flavor)
- 10) 4-carvomenthenol (mint flavor)
- 11) 5-hydroxymethylfurfural (wood flavor)

Figure 8a-7. Chromatogram of Peppermint Schnapps.