LOOKING FOR TOXIC METHANOL

Trace the silent killer in alcoholic drinks

In 2012, the Czech Republic was paralyzed by „The methanol affair“. Some manufacturers of alcoholic drinks at black market, to avoid of paying the high consumption tax, added methanol into the alcoholic drinks instead of ethanol due to similarity in taste and smell. By this method, they prepared alcoholic beverages significantly cheaper. Unfortunately, the methanol is highly toxic compound. Their ignorance of chemistry and toxicology knowledge, however, was fatal. In consequence, more than 50 people died and much more experienced health problems or permanent damage of people health was observed. With the affair, also neighbouring countries were affected, especially Poland and Slovakia. In addition to that, several million bottles with alcoholic drinks with uncertain content remained at the market. To withdraw the bottles containing the toxic methanol, and also as evidence against the people who spread out the dangerous drinks with alcohol, the suitable analysis of presence (and content) of methanol (in presence of ethanol) was necessary. As the most important method, a gas chromatography has been employed.

Become a member of specialized team on investigation of methanol affair! Analyze a sample with unknown mixture and find out whether there is the toxic methanol. Help to investigators trace people responsible for methanol affair. You are an expert with necessary knowledge and you should analyze unknown sample from store of suspect using one of the most important method of forensic analysis.

What you should to know?

Problem of detection and analysis of methanol is concurrent presence of ethanol in samples of alcoholic drinks. Both the alcohols are very similar, as well as in physical and chemical properties, because the alcohols differ by one CH_2 group only. For majority of analytical methods, this similarity causes practically impossible to analyze them in mixture. Fortunately, there is a method which is able to distinguish between the alcohols and analyze them together. The name of the method is the gas chromatography, which is also one of the basic methods of forensic analysis. Maybe, you already heard about „chromatography“. What is it?

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What you should know?

There is a variety of chromatographic techniques, and, probably, you know some of theme. You heard about paper chromatography, thin-layer chromatography (TLC), liquid chromatography, high pressure liquid chromatography (HPLC) and … gas chromatography (GC). The employment of chromatographic techniques is very wide. They are used in biochemistry for identification and separation of proteins, in biology for identification of plant dyes. Organic chemists use GC for control of synthesis, analytical chemists for analysis of pollutants in environment, drug analysis, etc. Chromatography is also used, as mentioned, in forensic methods.

All the chromatographic techniques are based on the same principle. Analyte (analyzed mixture or compound) is dispersed in so called mobile phase. The mobile phase goes through stationary phase, which is usually in column or bar, and carries the analyte through the stationary phase as well. The analyte interacts with mobile phase as well as with stationary phase and a lot of equilibriums are established in the framework of these interactions. Some compounds in analyte interact more with stationary phase, some of them have stronger interactions to mobile phase, in some cases the interactions are almost equal. The difference in interactions causes that compounds with stronger interactions to stationary phase are retained in the column and travel more slowly than the compounds with stronger interactions to mobile phase. The consequence of the mentioned interactions and different speed of compounds in the column, if the column is long enough, the compounds are separated. The interactions of compounds are mostly based on dipole-dipole interactions, disperse interactions and ionic interactions.

Our chromatograph GC Mini uses a metal column which is inside covered with special stationary phase using modified siloxane. Sample (mixture of ethanol and methanol) is injected into the column and the volatile compounds in the sample are evaporated and carried by mobile phase (air in our case) into the column. In the column, the components of the mixture are separated. The components reach the detector at different times, as they are differently retained in the stationary phase. In the detector, they are ionized which changes or causes some change of current in the detector which is consequently converted to voltage measured by the system. The individual compounds are represented at chromatogram (voltage as a function of time) as “peaks” (Fig. 1). The time necessary for the individual compound to pass the column is called *retention time* and this time characterize the compound (at given stationary and mobile phase and system parameters – temperature, pressure, mobile phase flow, …).

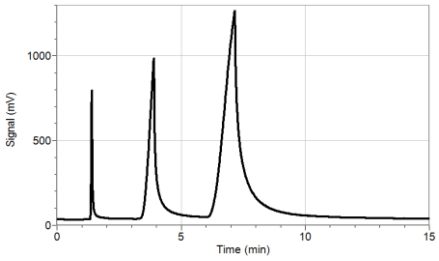


Figure 1: Typical chromatogram (GC)

There is a variety of factors influencing movement and interaction of compounds with the stationary and mobile phase. More volatile compounds usually go quicker through the column as their interactions with stationary phase are weaker. The presence of functional groups is another important factor for separation. For example, the alcohols, they have a dipole moments and, hence, they better interact with polar stationary phases and less with usually non-polar mobile phase than, for example, in the case of ethers with smaller dipole moment and negligible ability to form H-bonds. There is also an influence of molecular weight, although, we cannot generally say that heavier molecules go more slowly through the column.

In the case of analysis, there are few ways how to identify and quantify the compound. The easiest way is, at given and constant conditions, to measure the retention times of standards expected in the analyte. Then, we measure a chromatogram of sample analyzed. Finally, we compare the retention times of analyte and standards. The same retention time indicates that analyte is the same compound as the corresponding standard.

Procedure

Chemicals and equipment:

- Gas chromatograph Vernier GC mini
- Standards - ethanol and methanol in vials with septum,
- Computer with Logger Pro 3 software or Labquest datalogger
- 1 mL syringe with thin needle
- Sample (analyte) in vial with septum

Before you start …

Try to fill in the table below and try to estimate the order of the compounds with respect to their retention times. You can use internet to identify the boiling points of the compounds.

Compound	Boiling point (°C)	Order
Methanol		
Ethanol		
propanol		

Procedure

Remember your safety. Take glasses, lab coat and gloves. Perform the experiment in fume hood or in well ventilated room.

Important: In the case you use a glass syringe (which is fragile), be careful. Do not bend the syringe, its plunger or needle. Do not remove the plunger from the syringe.

Prepare the chromatograph for measurement.

a. Switch on the Mini GC chromatograph.
b. Connect GC Mini via USB cable to computer or datalogger.
c. Run Logger Pro software (or use Labquest), select New in menu "*File*"
d. Select "*Collect*" and the item "*Temperature-Pressure profile*".
e. Set on the following "*Temperature-Pressure*" profile:

Start temperature	60 °C
Hold time	3 min
Ramp rate	5°C/min
Final temperature	60°C
Hold time	3 min
Total length	6.0 min
Pressure	1.0 kPa

f. Select "*Done*" – GC Mini starts heating of the column. Note: New message appears "*Do not inject until GC is ready*", a LED diode of GC Mini is red. In few minutes, the column of GC Mini is heated to the selected temperature. The GC Mini is ready for sample injection – see later how to inject the sample – message "*Inject and select Collect simultaneously*" appears – Inject the sample and simultaneously select "*Collect Simultaneously*", a LED diode is switched from red to green. In the course of

heating of the column, you can make the cleaning of the syringe and intake of the sample for injection:

To clean the syringe, use following steps:

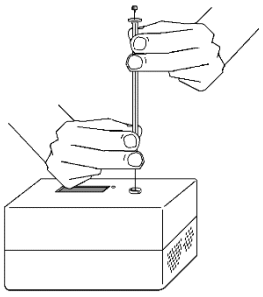
a. Insert fully the plunger.
b. Intake and deflate air 5 times and consequently, do this 5 times with cleaning liquid (acetone, methanol, …).
c. Deflate carefully the sample into pulp.

Preparation – standard or sample – WARNING!!! – if you inject vapor or gas, you must not intake a liquid into the syringe (or drops of the liquid)!!!

a. Insert needle of syringe into vial with standard (or with sample) through septum to insert the needle into liquid in vial (there is less than 40% of vial volume filled in).
b. Turn the vial around. The needle must be now ABOVE the surface of liquid in the vial (more than 5 mm).
c. Several times insert and pull out the plunger to intake the vapors in vial. Avoid intake of liquid in the vial (if you inject the liquid into GC Mini, you can brake it).
d. Intake ca 1,3 mL of gas (vapor) above the liquid in vial. Turn the vial around to initial state and take the syringe out.
e. Move the plunger to have the volume of vapor in syringe of 1 mL.
f. Gently wipe the needle over pulp.

Prepare to inject the standard or sample into chromatograph. Inject the sample immediately after you intake the vapor (but the GC Mini must be ready for measurement, so, plan well your work). It is advantageous to work in pairs. The first member control the chromatograph, the second member is responsible for sample injection.

a. If GC Mini reached the selected temperature of the column and selected pressure, it is ready for sample injection. The message "*Inject and select Collect simultaneously*" appears and LED diode is green.
b. Insert needle into hole with septum at chromatograph. Hold the syringe with hands, vertically, one hand at upper part, and the second hand on bottom part of the syringe (Fig. 2). Do not use plunger for now.
c. Simultaneously, press plunger and click on "*Collect*". Remove the syringe carefully.



Obr. 2

In the course of measurement, rinse the syringe with air several times (intake and insert the plunger).

Data collection is finished in ca 6 minutes.

Analyze your chromatogram

a. Select "*Peak Integration*" from menu "*Analyze*"
b. Select the peak for integration (go to left side of the peak, press the left mouse button and hold it. Move the mouse to the right side of the peak. Release the left mouse button. Select "*Add*."
c. Write down the retention time (into the table below) and other information.
d. In the case of presence of more peaks in chromatogram, analyze all of them.
e. When all peaks are selected and analyzed, press OK.

You can also save the chromatogram and analyze later. Just select item "Save" in menu « File ».

Make the mentioned procedure with all the standards and with sample as well.

Evaluate and interpret your measurements and results.

When finished, switch the GC Mini off.

Data evaluation

Fill in the table with measured data.

Compound	Boiling point (°C)	Order (1-3)	Retention time (min)
methanol			
ethanol			
propanol			

Sample analysis

Peak	Retention time

Answer the following questions

1. In the table above, you made an order of alcohols measured. Were you right? Why? Were you lucky or your prediction was based on some knowledge?

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2. On the basis of comparison of chromatograms of standards and chromatogram of sample, give an answer - which alcohols are present in the sample from investigators? Is the toxic methanol in the sample, so as, is the drink dangerous?

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3. Identify alcohols in another sample. Why do you think that the alcohols are in the sample?

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Conclusion

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Communicate your results

Write and send to investigator official results (report) about results of your analysis. In advance, do not forget to state what parts and what kind of information must be included in the report (hint: used method, column, …).

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Specific questions

1. Predict and explain the retention time of butan-1-ol.

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2. Which compound can be analyzed with gas chromatography? Why?

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