GC/MS Determination of Caffeine in Beverages

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Abstract

A simple and quick procedure has been tested for the GC/MS determination of caffeine in beverages. The procedure involves the mixing of 1mL sample with total 1.5mL ethyl acetate and 0.2mL vanillin, and a following simple drying procedure with sodium sulfate. A linear calibration curve was generated with caffeine concentration ranging from 50mg/L to 400mg/L. Vanillin was used as internal standard and all caffeine solutions and samples were spiked with vanillin. The linear calibration curve had R^2 value of 0.9949 but the caffeine extraction procedure for the GC/MS determination of caffeine in beverages requires further study.

Introduction

Caffeine, a naturally occurring alkaloid, is added to many soft drinks and energy drink as a flavoring agent.[1] With a large number of people consuming such beverages on a daily basis, it is important to recognize and limit the amount of caffeine intake for health reasons. Excessive caffeine intake can lead to a fast heart rate, nausea, vomiting, anxiety, depression and insomnia.[1] While the US Food and Drugs Administration regulate the caffeine levels of soft drinks to a maximum of 200mg/L, there is no legal limit for caffeine levels for energy drinks.[1]

Since soft drinks are regulated in how much caffeine they can contain, caffeine analysis is often requested to ensure proper caffeine levels in beverages and to meet regulatory standards. There are several methods for caffeine determination in soft drinks: UV spectroscopy, HPTLC, capillary electrophoresis, HPLC, and *etc.*[1] In this experiment, GC/MS was used to determine the caffeine level in beverages.

Principle of Method

GC-MS is a combined analytical technique in which gas chromatography and mass spectrometry are combined (Figure 1).[2] GC-MS is a technique in which components mixtures can be separated chromatographically and then detected by mass spectrometry.[2]

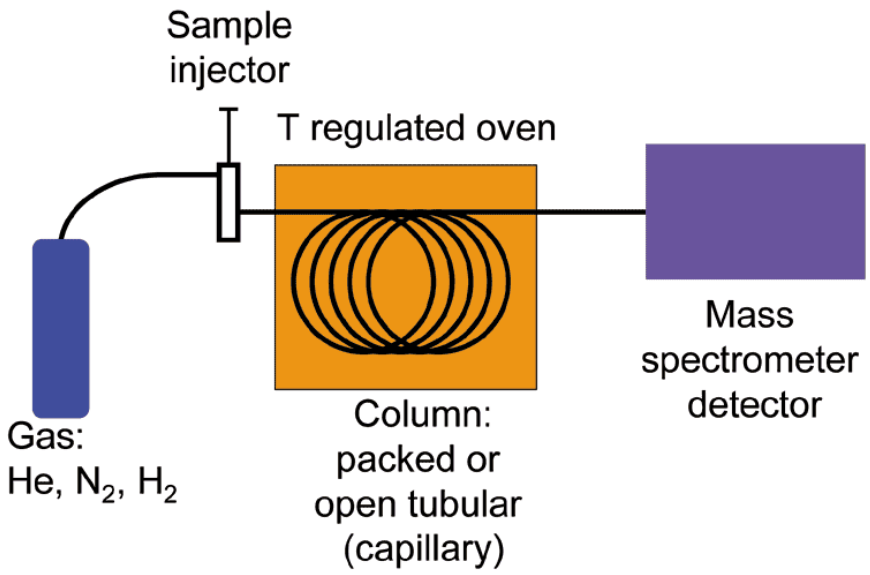

Figure 1. GC-MS schematic

First, the sample is introduced to gas chromatography in order for the analyte to be separated.[2] Initially, the GC part of the GC-MS is set with 100°C oven and with the injector with 1mL/min of helium flowing through the injector into a capillary chromatographic column. After the sample has been injected to GC, the sample is first vaporized in the 100°C injector for the first 1 minutes. The semi-volatile analytes concentrate at the head of the 100°C column. Then the injector starts allowing helium flow to flush residual solvent and analyte from the injector at 1mL/min. The oven temperature starts ramping at a rate of 20°C/min. As the temperature starts ramping, the low boiling solvent, ethyl acetate in this case, elutes first. After air and solvent have fully eluted from the column, the mass spectrometer is turned on and the mass to charge ratio from 75 to 300 are monitored. As the temperature continues to increase in the column, analytes elute sequentially in the order from the analyte least interacting with the column to the analyte most interacting with the column. The analytes are detected by the MS in GC-MS and processed.

There are several ways for MS data to be processed and output. In reconstructed ion chromatogram (RIC), a plot of the summed totals of the counts for all ions in each mass spectrum vs. time is obtained.[2] In extracted ion chromatogram (EIC), plots of counts vs. time using data selected at m/z ratios unique to particular analyte ions are obtained.[2] Mass spectra are

plots of counts vs. m/z ratios of the ions of the eluted compounds providing qualitative information about the analytes.[2] In this experiment, an RIC chromatogram is plotted.[2]

Once all analytes have been detected, the oven temperature of GC reaches 250°C and the run terminates. The MS is turned off and the oven temperature is cooled back to 100°C for the next run.

The GC-MS used in this experiment is equipped with a capillary chromatographic column to separate the analytes.[2] The carrier gas is helium to transfer or carry the analytes through the column.[2] Analytes elute directly from the column into the cavity of an ion trap mass spectrometer (Figure 2).[2] The cavity is a ring electrode and two cap electrodes.[2] A 70eV electron beam from a heated filament ionizes the molecules from GC.[2] The ions initially are trapped in the cavity by the field of a radio frequency (RF) storage voltage applied to the ring electrode.[3] The RF voltage is ramped such that the ions, in order of increasing m/z ratio oscillate axially towards the cap electrodes.[3] At critical RF voltages, the ions are ejected sequentially in order of increasing m/z ratio through holes in the bottom end cap where they are detected by an electron multiplier.[3] The MS part in GC-MS is set to have 10^{-4} to 10^{-8} torr pressure to create vacuum which reduces chance of interaction with the atmosphere with the ionized samples.[3]

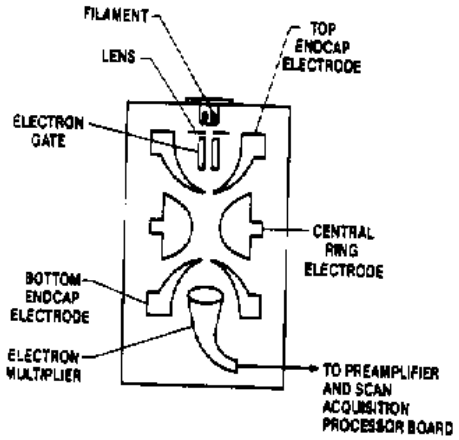

Figure 2. Ion Trap detector schematic[4]

An electron multiplier is usually constructed with a high vacuum inside, which contains a photocathode, dynodes, and an anode (Figure 3).[3] Incident ion beam strikes the photocathode

material and the electrons which have been deposited on the thin surface of the photocathode, get kicked out due to the energy of the ion beam.[3] As the ejected electrons move toward the first dynode, they are accelerated by the electric field and gain much greater energy.[3] Once the electrons strike the first dynode, more low energy electrons get emitted and these electrons are accelerated toward the second dynode.[3] This process repeats several times and eventually the number of emitted electrons increases amplifying the signal.[3] The signal is stored, processed, and counted as a function of m/z.[3]

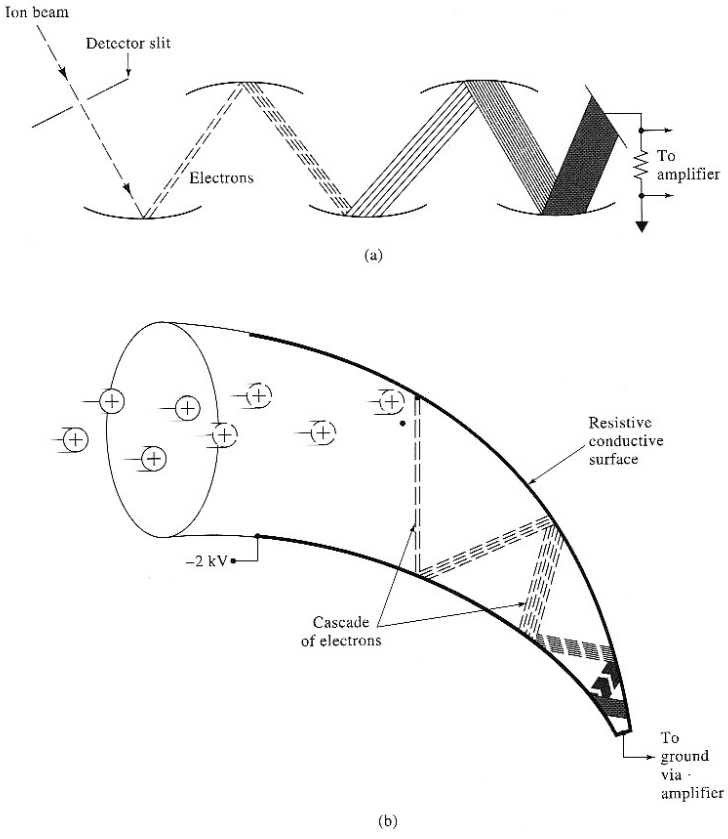

Figure 11-2 (a) Discrete dynode electron multiplier. Dynodes are kept at successively higher potentials via a multistage voltage divider. (b) Continuous dynode electron multiplier. *(Adapted from J. T. Watson,* Introduction to Mass Spectrometry, *p. 247. New York: Raven Press, 1985. With permission.)*

Figure 3. Electron Multiplier schematic[3]

The objective of this experiment is to determine the caffeine from the comparison between the known and unknown solutions of caffeine. In order to do that, internal standard vanillin is added to all solutions. The presence of the internal standard allows correcting for the

loss of analyte during sample preparation, sample inlet or injection.[2] Since the same amount of internal standard is added to all standard solutions, if all conditions were ideal, the internal standard's area should roughly be the same. The internal standard method provides a calibration curve of the ratio of the analyte signal to the internal standard signal vs. the concentration of the standard solutions.[2] Using the calibration curve, the concentration of unknown caffeine can be determined.[2]

Experimental

Standard Solution Preparation

The standard solutions were prepared with caffeine concentration ranging from 50mg/L to 400mg/L. They were all prepared in 10mL volumetric flask with ethyl acetate. 2500mg/L caffeine stock solution was used to prepare the standard solutions. Vanillin stock solution was prepared with the concentration of 5000mg/L and 0.120mL of the vanillin solution was added to each of the standard solutions.

Sample Preparation

The samples in this experiment include Coca Cola, Diet Coca Cola, and Red Bull. 5mL of beverage sample was taken in a small beaker and the beaker was sonicated to degas the sample. 1mL of a sample was transferred into a small vial and 500µL of ethyl acetate was added. The vial was inverted gently a few times and the mixture was allowed to separate into two layers. The top ethyl acetate layer was transferred to a clean small vial. The above procedure was done three times so that there will be total of 1.5mL of ethyl acetate in the small vial. 200µL of 1000mg/L vanillin solution was added into the vial and 0.10g of sodium sulfate was added to remove any water from the extract.

Instrumentation

The GC/MS analysis was carried out on a gas chromatograph with a capillary chromatographic column and a mass spectrometric detector equipped with an electron ionization source and an ion trap detector. Analytes were separated on a capillary column and the helium carrier gas was set at a constant flow-rate of 1mL/min. Samples were injected using headspace method, drawing up 100 – 200µL of gas touching the surface of a sample solution with 250µL syringe and injecting the gas into the GC. The 250µL syringe was washed at least three times

with ethyl acetate gas before and after each run and rinsed with sample solution gas before the sample gas was injected. In order to make sure that there is no contaminant in the column and syringe, several blank runs were performed with and without syringe injection. Sample injection was done after a blank run with and without blank syringe injection showed no significant peak in the chromatogram. The split ratio of GC/MS was 20:1 and the initial temperature of the GC oven was 100^{o}C. The GC/MS instrument was run at 100^{o}C for the first 1 min and the oven was ramped at a rate of 20^{o}C/min to 250^{o}C and held at this temperature for 1 min. Solvent delay was applied for the first 2 minutes to remove high ethyl acetate peak.

Results and Discussion

Chromatogram of caffeine and vanillin

Chromatogram from GC/MS analysis shows strong peaks at 5 min and 8 min (Figure 5). The MS analysis of the peak at 5 min shows a strong abundance of an ion with *m/z* = 152 and the peak at 8 min have a strong abundance of an ion with *m/z* = 194 (Figure 4). Thus, the peak at 5 min must be from vanillin and the peak at 8 min is from caffeine since vanillin and caffeine have molecular mass of 152 and 194 respectively. However, the position of the peaks may be doubtful if one considers the boiling point of vanillin and caffeine. Vanillin has a boiling point of 285^{o}C and caffeine has a boiling point of 178^{o}C. According to the boiling point of vanillin and caffeine, caffeine is more likely to be eluted earlier than vanillin since caffeine will be at vapour state earlier than vanillin. The chromatogram of caffeine and vanillin in this experiment shows that the retention time of a compound does not only depend on the boiling point of the particular compound. The retention time of a compound also depends on the compound's degree of interaction with the column and volatility. In this experiment, boiling points of caffeine and vanillin do not contribute significantly to the retention time of each of the compounds since caffeine and vanillin were already at a gas state during the injection. The reason why vanillin was eluted earlier than caffeine was is because vanillin is much more volatile than caffeine. Pure vanillin compound exhibits strong odor meaning that vanillin molecules are fast moving while caffeine compound does not have a noticeable odor. Since vanillin molecules can move faster than caffeine molecules, it is understandable that vanillin molecules went through the column faster than caffeine molecules. Since all sample solutions were prepared in ethyl acetate, an extremely volatile solvent, solvent delay was applied for the first 2 min for better analysis of

caffeine and vanillin peaks. Ethyl acetate peak appeared around at 1.5 min when there was no solvent delay.

Headspace injection & peak intensity

Since the GC/MS instrument used in this experiment is shared, headspace injection was used in order to avoid any column overloading. The strength of headspace injection is that column overloading can be prevented by injecting a small amount of analyte gas into the column. However, there is also a weakness in headspace injection. If the analyte is not volatile enough, a very small amount of analyte molecules would be injected and the detector would not be able to detect the analyte. Even if the analyte is detected, there is a high chance of having low signal to noise ratio due to low detection level. Fortunately, vanillin and caffeine were volatile enough to use headspace injection. However, it was difficult to obtain peak intensity that is high enough for decent signal to noise ratio. Thus, the injection volume was adjusted from 100 to 200µL to obtain peak with abundance of at least 10000.

Calibration curve of standard solutions

The strong molecular ion peak of caffeine at *m/z* 194 and vanillin at *m/z* 152 were selected as the quantitative signal. The calibration curve was constructed by plotting the ratio of measured peak areas of caffeine and vanillin versus concentration of caffeine. Excellent linearity was obtained within the caffeine concentration range of 50 – 400 mg/L, giving the R^2 value of 0.9949 (Graph 1). There is also an error in the calibration curve as the y-intercept is non-zero, 0.1106 (Graph 1). The possible experimental error could be from the integration for peak area. The range of integration and the background noise can change the integration value for peak area significantly. Moreover, there is always an issue with the preparation of standard solutions. As the concentration measured in GC-MS increases, there is a higher chance for ions in the ion trap detector to interact with each other. This can certainly cause significant error as the ion counts would be influenced by the interaction between the ions.

Caffeine extraction of beverage samples

Caffeine level in an unknown solution can be determined using the calibration curve constructed by plotting the ratio of measured peak areas of caffeine and vanillin versus concentration of caffeine. The calibration curve gives a linear line with an equation,

$y = 0.0225x + 0.1106$ where y is the ratio of caffeine peak area and vanillin peak area and where x is the concentration of caffeine in the solution (Graph 1). Hence, the caffeine concentration of analyzed solution can be determined by solving x in the equation,

$x = \frac{y - 0.1106}{0.0225}$. Since the injected sample has been diluted, x is the concentration of the diluted solution. In order to determine the original caffeine concentration of a sample, x has to be divided by the dilution factor. For example, in this experiment, 1mL of a beverage sample was used and diluted to 1.7mL as the sample was extracted with total 1.5mL of ethyl acetate and as 0.2mL of vanillin solution was added after the extraction. Thus, the original caffeine concentration of a beverage sample would be $[original\ caffeine] = \frac{x * 1.7}{1}$.

Before beverage samples were prepared, a trial sample had been prepared in order to check whether the extraction procedure works or not. A 100mg/L caffeine solution was prepared with deionized water as the caffeine level in coke is around 100mg/L. The caffeine solution was extracted using the procedure explained as above and injected using headspace method. The GC/MS analysis of the extracted caffeine sample gave a vanillin peak at 7.5 min and a caffeine peak at 11 min (Figure 6). The retention times of caffeine and vanillin were different because different GC parameter was used in which the initial temperature of the column was 75°C for 2 min and was ramped at 20°C to 200°C. However, when beverage samples were extracted and prepared with the same procedure described as above, there is no peak showing in the chromatograms. A possible explanation to this failure is that the concentration of the beverage samples is too low for GC/MS to detect the analytes. However, GC/MS could detect caffeine and vanillin in a standard solution with similar concentration as the beverage samples. In order to make GC/MS detect the beverage samples, the beverage samples were heated at around 60°C for 2 min making the samples more volatile. Heating the samples did not work well as GC/MS still could not detect the heated samples. The other explanation to GC/MS not being able to detect the beverage samples can be failure of the extraction procedure on beverage samples. Further studies are required for the extraction procedure as the procedure worked with a caffeine solution in

water but not with beverages. There is another simple procedure for the GC/MS determination of caffeine in beverages done by Zou and Li using 25µL of soft drink sample, 1mL of ethyl acetate, and 0.005g of magnesium sulfate.[1]

Conclusion

A simple and rapid procedure was developed for the determination of caffeine in beverages but further studies are required as the extraction procedure did not work with beverages but only with caffeine solution in deionized water.

Vanillin was proven to be an ideal internal standard for the GC/MS determination of caffeine. The calibration curve constructed by plotting the ratio of caffeine and vanillin peak areas versus caffeine concentration had a R^2 value of 0.9949.

Reference

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