UNC PEMBROKE | MAYNOR HONORS COLLEGE

Rapid Determination of Caffeine in Beverages using Mass Spectrometry and an Atmospheric Solids Analysis Probe

Senior Project

In partial fulfillment of the requirements for
The Esther G. Maynor Honors College
University of North Carolina at Pembroke

By

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Abstract

Caffeine is a common ingredient in multiple beverages and is either naturally present or intentionally added as a stimulant. The concentration of caffeine must be known and then regulated for consumer safety. The preferred method for quantifying caffeine in beverages uses high performance liquid chromatography (HPLC) to both separate and quantify the beverage ingredients. Atmospheric solids analysis probe mass spectrometry (ASAP -MS) is an analysis tool used primarily for identifying the components of liquid and solid mixtures. It requires little or no sample preparation and can make multiple rapid measurements. Our research goal is to develop an ASAP-MS procedure that is a simple and fast alternative method to both identify and quantify caffeine in beverages. ASAP -MS in positive ion mode (ASAP (+)- MS) was optimized for detection of caffeine (CAF) in different beverage samples with acetaminophen (ACE) added as an internal standard to permit precise quantification. Optimization experiments determined that use of an ion source gas at 300 °C and selected ion monitoring at 195 amu (CAF) and 110 amu (ACE) resulted in the highest quality signals. Standards and spiked beverage samples were directly analyzed with minimal sample extraction techniques. In this work, we present the results of experimental studies that suggest ASAP (+)- MS may be a suitable method for rapid quantification of caffeine in beverages. This work was performed in partial fulfillment of requirements for graduation from the Esther G. Maynor Honors College.

Rapid Determination of Caffeine in Beverages using Mass Spectrometry and an Atmospheric Solids Analysis Probe

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ABSTRACT: Caffeine is a common ingredient in multiple beverages and is either naturally present or intentionally added as a stimulant. The concentration of caffeine must be known and then regulated for consumer safety. The preferred method for quantifying caffeine in beverages uses high performance liquid chromatography (HPLC) to both separate and quantify the beverage ingredients. Atmospheric solids analysis probe mass spectrometry (ASAP -MS) is an analysis tool used primarily for identifying the components of liquid and solid mixtures. It requires little or no sample preparation and can make multiple rapid measurements. Our research goal is to develop an ASAP-MS procedure that is a simple and fast alternative method to both identify and quantify caffeine in beverages. ASAP -MS in positive ion mode (ASAP (+)- MS) was optimized for detection of caffeine (CAF) in different beverage samples with acetaminophen (ACE) added as an internal standard to permit precise quantification. Optimization experiments determined that use of an ion source gas at 300 °C and selected ion monitoring at 195 amu (CAF) and 110 amu (ACE) resulted in the highest quality signals. Standards and spiked beverage samples were directly analyzed with minimal sample extraction techniques. In this work, we present the results of experimental studies that suggest ASAP (+)- MS may be a suitable method for rapid quantification of caffeine in beverages. This work was performed in partial fulfillment of requirements for graduation from the Esther G. Maynor Honors College.

A recurring theme in research aimed at developing new methods for chemical analysis is to decrease as much as possible both the quantity of sample and the time required to perform the analysis. A problem that arises when using methodologies with little sample consumption is the low signal strength, decreased measurement precision, and lower detectability of sample components[1]. Reducing the sample quantity without sacrificing the integrity of the analysis is a challenged faced by many scientists working to develop new methods of chemical analysis.

Caffeine is a stimulant to the central nervous system and has been long used as a method to stay awake and alert. Caffeine is a naturally occurring compound found in over 60 plants but used as a pesticide to prevent some insects and animals from destroying it. Caffeine and its stimulant effects have been known for a long time and is known said to be the "most frequently consumed psychostimulant worldwide"[1]. Common caffeine containing beverages include tea, coffee, soft drinks, and energy drinks. The Food and Drug Administration (FDA) recognizes caffeine as a safe substance whose levels should not exceed 200 parts per million. Energy drinks have become a leader of providing large amounts of caffeine to the public, up to 300 mg/bottle in certain drinks. While caffeine is technically regulated by the FDA, there are different standards depending on the substance such as food, drink, dietary supplement. The FDA does not require dietary supplement marketers to state their caffeine contents on the label, so many energy drinks will classify themselves as dietary supplements. Those dietary marketers do not need preapproval from the FDA to put caffeine and other certain ingredients in their supplements[2].

There are many different techniques that can be to measure the amount of caffeine in beverages, such as thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and others. Analyses using these techniques often require relatively long analysis times and complicated sample preparation procedures. For example, one HPLC method used for the quantification of caffeine, along with other substances, in drinks was reported to take a total of 55 minutes due to the long retention times of the various beverage ingredients[3]. In comparison, the use of an atmospheric solids analysis probe coupled with a mass spectrometer (ASAP-MS) enables the collection of data in a matter of seconds, requires little or no sample preparation, and can accommodate very small sample volumes[4]. The precision of the ASAP sample introduction procedure, however, is inherently poor and so it is typically restricted to qualitative analyses, being poorly suited for quantitative applications[5].

The study described here explores the use of ASAP-MS for the rapid quantification of caffeine in beverages. To compensate for the inherently poor precision of the ASAP sample introduction technique, an internal standard calibration scheme was employed in which known amounts of a reference substance, acetaminophen, was added to all samples.

EXPERIMENTAL

Preparation of Reagents. The first solutions prepared were done so to provide approximate concentrations of 100 ppm. Deionized water was used as the solvent and 0.1002 g of pure caffeine was dissolved in 1 liter of the deionized water using a graduated plastic chemical storage bottle. This sample was used as the caffeine standard of known concentration. When not in use the bottle was stored in the fridge until the next use. Another sample was prepared that contained 100 ppm caffeine and 100 ppm acetaminophen (ACE). A mass of 0.1004 g ACE was dissolved in the liter of 100 ppm caffeine solution. The sample was used to observe how the two analytes interact and perform with the ASAP-MS method. When ready for analysis the solutions are transferred into smaller centrifuge tubes that are partially filled to a certain depth in which the probe can be inserted and collect sample while also not allowing the liquid to get in contact with the brass head. The insertion of the probe was done manually so the centrifuge tubes were filled to a certain height that would allow the probe to be fully inserted without contact to the brass base. Keeping the insertion depth as constant as possible allows for better, more reproducible data.

An ASAP-MS instrumentation tuning solution was provided (Agilent Technologies) that consisted of a mixture of different standard compounds and was used prior to use of the MS to ensure the instrument was working properly and reading the correct masses.

Instrumentation. The mass spectrometer used was an Advion Expression[s] CMS configured with an atmospheric pressure chemical ionization (APCI) source, a quadrupole mass analyzer, and a channel electron multiplier detector. Samples were introduced using an atmospheric solids analysis probe (ASAP). Signal acquisition was accomplished using the Advion Data Express software application.

Procedure. The ASAP glass capillary probe is contacted with the sample to ensure that the sample will be introduced to the instrument, in this case the tip of the probe was dipped into the solutions. The sample is introduced to the mass spectrometer by inserting the probe into the ASAP housing. Heated nitrogen gas vaporized the sample, and the corona discharge needle served to ionize the gaseous sample components (see Figure 1). Data were typically acquired continually during repetitive sample insertions.

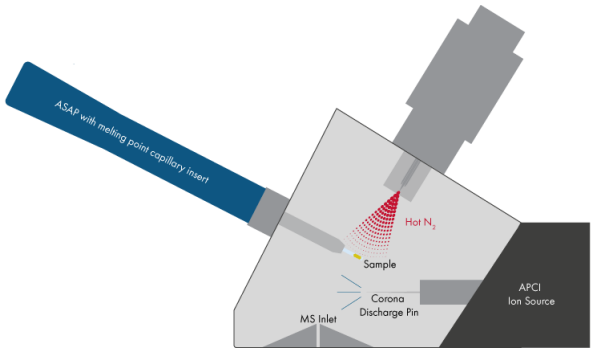

Figure 1. Schematic illustration of the APCI source with ASAP probe. The stream of hot gas vaporizes the sample and the corona discharge needle ionizes the vaporized sample before entering the mass spectrometer. Image courtesy of Advion, Inc."

Data Analysis. Acquired data were analyzed using the Advion Data Express software application. It is an easy-to-use data processing software program that operates with the Advion Mass Express to analyze the mass spectral data. It has the capability to quickly and automatically detect significant peaks within the mass spectra and calculate their absolute intensities and integrated areas. Measured parameters important for this study included the maximum intensity, peak area, %peak area, peak resolution, and the peak mass. These parameters were subjected to statistical analyses using MS Excel.

The statistical calculations that took place during this laboratory included finding the average value, the standard deviation, and the coefficient of variation (CV)/ percent relative standard deviation (%RSD). To find the average peak area the following equation was used:

$$\bar{x} = \frac{\sum(x_{peak\ area})}{n}$$

Where x bar is equal to the peak average, x is the peak average for each run, and n is the number of runs.

To calculate the standard deviation (σ) of the peaks at a certain temperature the following formula was used:

$$\sigma = \sqrt{\frac{|x - \bar{x}|^2}{n}}$$

To calculate the CV or %RSD the following formula was used:

$$CV = \frac{\sigma}{\bar{x}} \cdot 100\%$$

The coefficient of variation (CV) was calculated along with the average area under the peaks and the standard deviation of those areas. The CV shows the amount of variation around the mean/ average value.

RESULTS AND DISCUSSION

Selected Ion Monitoring. The original signals were acquired in mass-scanning mode, measuring mass spectra over a broad range to help in identification of the peaks that will be of interest in the experiment. A 100 ppm sample of caffeine and a 100 ppm sample of ACE were each analyzed at 300-degree gas temperatures and the two signals were compared to each other. This comparison was done to identify peaks of interest for each analyte. The prominent mass spectral peaks in caffeine were found to be at 139, 167, and 195 m/z (largest signal). The prominent peaks for acetaminophen were found to be at 110 (largest signal) and 152 m/z. In order to gain greater quality peaks with higher signal to noise ratios, the mode of acquiring the signals was changed from measuring a wide mass range to only looking at the selected ions. The selected ion monitoring (SIM) will be set to show the data collected for the two analytes only by observing the signals at 195 m/z for caffeine and 110 m/z for acetaminophen. Reference electron ionization chromatograms were used to confirm this SIM ion selection from the National Institute of Standards and Technology[8]. These supported chromatograms did display the masses as one value lower than the Advion Expressions CMS because it uses a different method of achieving the signal. With confirmation of the two prominent peaks, the lab experiment will be performed using the selected

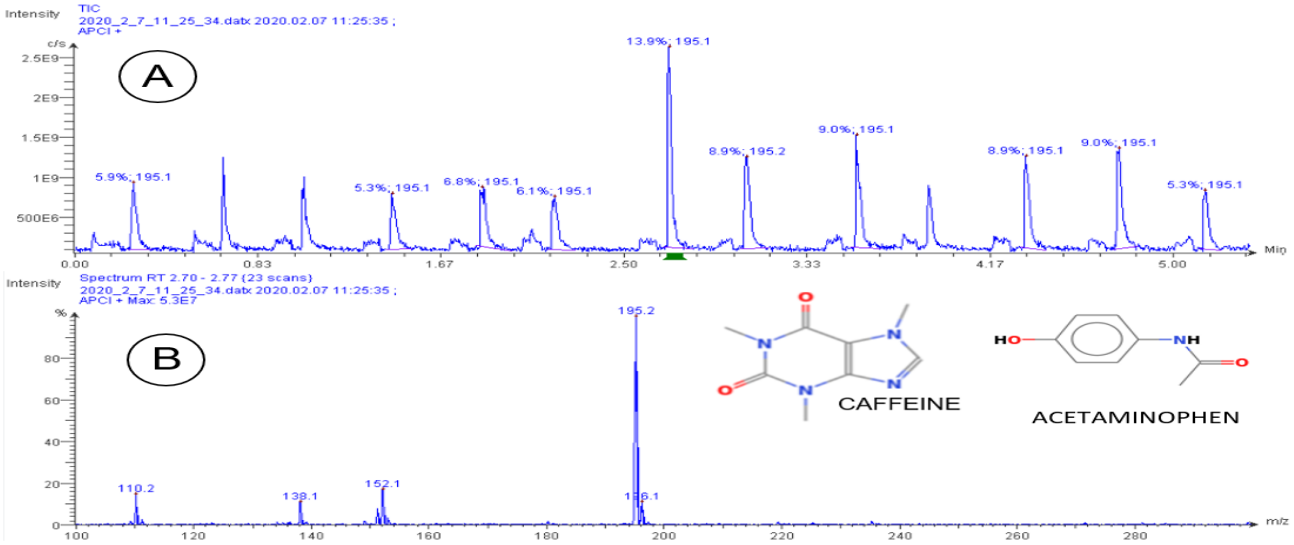

Figure 2. Signals for 100 ppm CAF and 100 ppm ACE TIC at 300 °C and mass spectrum. (A)The TIC demonstrates the lack of reproducibility in using the ASAP-MS method. Each peak represents a new introduction of sample into the instrument and the TIC shows that the signal intensity continues change with each insertion. (B)The mass spectrum identifies mass to charge ratios present in the sample. The main components masses are identified in the mass spectrum (110, 138, 152, 195 m/z). There is a chemical structure of CAF an ACE provided in figure

ion monitoring for 110 m/z and 195m/z. The data collection for each of the temperature settings will be done under SIM and the peak areas for CAF and ACE will be analyzed and statistically compared.

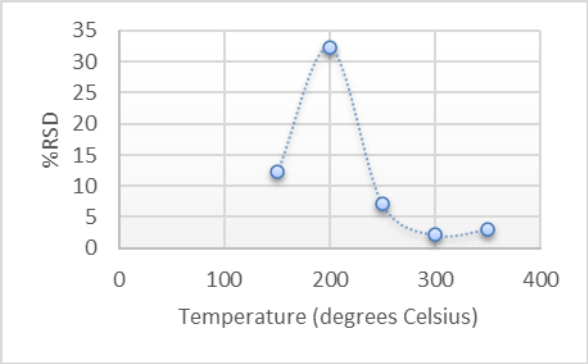

Figure 3. %RSD (n=12) versus capillary inlet temperature in degrees Celsius. The %RSD values were those for the peak signal ratios (internal standard approach).

Internal Standard. The ASAP-MS mechanism has poor precision, and this was proven by performing a repeat of insertions (12) of a known standard of caffeine that contained 100 ppm of caffeine in deionized water. The results in the total ion chromatogram showed inconsistency in the signal strength and there was an inability to provide one constant value for the standard solution (see Figure 2). To solve the lack of precision in the instrumentation and internal standard will be used. An internal standard of ACE is used for this laboratory experiment to account for the lack of reproducibility in probe insertion method. ACE will provide a mass spectrum reading that will differ from caffeine so that the two masses are distinguishable and can be used to set up ratios of peak are or signal. That is because no two measurements will be equal in signal strength since the vaporization of the substance on the probe can vaporize and be ionized in different quantities within the instrument for each mass spectral measurement. The internal standard of acetaminophen will provide an additional peak to observe that should be proportional to the CAF peaks of each measurement. This will provide reproducibility in the data to an accurate ratio of the peaks. Taking multiple signal readings of the sample of 100 ppm caffeine and 100 ppm ACE solution resulted in a multitude of different signal intensities ranging from less than 1.5x 10^8 c/s to 3.2x 10^8 c/s, that is a difference on the order of 1,700,000,000 c/s (Figure 4). The order of magnitude in signal when using a ratio of the signal of CAF to ACE is significantly smaller than the original signal of only CAF, on the order of tenths of a difference, signifying the advantage of using an internal standard to create more precise ASAP analysis. Using the ability to calculate the ratio of peaks and the %RSD value for ratios, it makes it possible to conclude which inlet temperature would provide the user the lowest %RSD value (Figure 3).

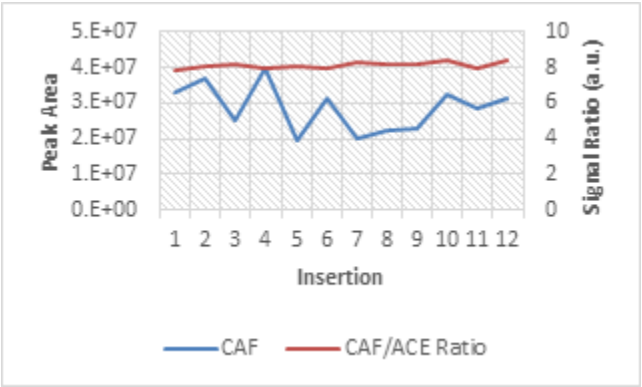

Figure 4. CAF peak area signal and the CAF/ACE ratio of signal for 12 insertions. The CAF signal varies greatly from each insertion from 1.5x10^8 up to 3.2x10^8 but the peak ratios have a smaller differential that spans from 7.78 to 8.45 a.u. The linearity of the CAF/ACE ratio line is more constant and reliable.

Temperature Optimization. The temperature of the nitrogen gas stream used to vaporize samples can be changed by the user so that the best results can be produced. To find the optimal temperature, replicate measurements (n=12) were made for insertions of the standard mixture of 100 ppm CAF and 100 ppm ACE at four different temperatures: 150, 200, 250, 300, and 350 °C. The signal parameters derived from these measurements were statistically evaluated to yield average values and relative standard deviations (%RSD) for the absolute signals of each compound and the ratio of compound signals. The goal was to find the temperature setting at which the lowest %RSD value could be found. The %RSD value for the absolute, individual peak signals are relatively high, but per the internal standard approach, the precision of the signal ratio measurements was much improved (lower %RSD). The %RSD value increases as the temperature drops below 300 °C. The %RSD value for the ratio of peaks for the run done at 300 degrees Celsius was calculated to be 2.17% and the value for 250 degrees Celsius was found to be 7.11% and it continues to decrease with the temperature. At 200 degrees Celsius the %RSD value was equal to 32.24% and at 150 degrees Celsius it was equal to 12.218 but had the longest retention time for the ionization of the molecules. The measurement time for each insertion took over five minutes for the peak to return to the baseline, comparatively the time it took for the 300 degree Celsius. The additional run at 350 degrees Celsius was to check if the %RSD value could go any lower and they are relatively close, but 350 degrees was slightly more, at 3.04% than the 300 degrees. 300 degrees Celsius would be the optimum gas temperature to run this experiment at for the analysis of CAF and ACE together.

The goal of this work is to provide an ASAP-MS procedure that is a simple and fast alternative method to identify and quantify caffeine in beverages with use of an internal standard. The addition of ACE as an internal standard was necessary to improve of the precision of the instrument.

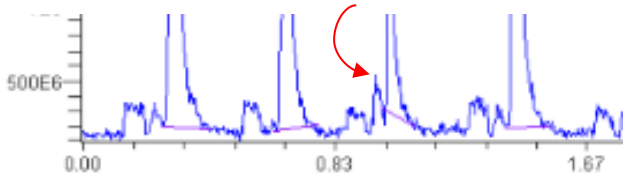

Figure 5. A signal/time trace for the 100 ppm ACE with 100 ppm CAF at 300 degrees Celsius zoomed in on the x-axis. The red arrow points to a peak that is seen to be the initial elution of acetaminophen, prior to the elution of the rest of the caffeine and ACE solution.

Differences in Vaporization Rates. One phenomenon that was experienced during this laboratory was the idea that acetaminophen vaporized from the ASAP probe more rapidly than the caffeine (Figure 5). In the signal/time traces there is evidence of an initial peak just prior to achieving the full intensity. The very first peak is attributed to the probe being absent from the instrument while obtaining the sample and then when the signal begins to rise again it sometimes reaches a point, decreases slightly and then completes the signal peak, it has an asymmetric shape (Figure 5). This was an interesting find and may be further investigated on another date. It was known that the ACE was the main component in that first part of the peak because in the Data Express analysis it was possible to isolate that peak and analyze the mass spectrum for that part of the peak. The contents of the beginning of the peak was mostly made up of the ACE showing a m/z of 110 and later on in the same peak the increase in elution of CAF at 195 m/z was occurring.

CONCLUSIONS

The experiment performed was done to provide an alternative method of rapid quantification of the caffeine in beverages using the ASAP-MS instrumentation. The issue at hand was the levels of caffeine in beverages and the concerns that they may bring to the consumers of those products. Since most energy drinks are not required to state the amount of added caffeine there is worry whether or not it is safe to consume. The purpose of this experiment was to use the ASAP and mass spectroscopy to develop a method that would provide one with precise results for the amount In the analysis it was found that, in order to obtain the most reproducible and precise measurements using the ASAP probe, an internal standard addition must be used. Acetaminophen was chosen as the internal standard since it could be distinguishable from caffeine by the mass spectrum and the caffeine and acetaminophen peaks could be turned into a ratio that remained constant throughout the collection of signal intensities and peak areas. Using acetaminophen as an internal standard will help in ensuring precision and reproducibility of the instrument analysis and help in quantifying the caffeine contents in beverages. This method and the optimization settings prove to be very promising, and analytically favorable for obtaining fast and simple quantifications of caffeine in beverages. In today's science community, the want and need for rapid determination and accurate analysis is growing and this procedure promotes just that with the use of an Atmospheric Solids Analysis Probe for introduction of the solution. Since heating of the sample takes place during the vaporization stage it is not safe to have extremely volatile samples for analysis with this technique. This experiment presents possible methods for future analysis of other solutions whose analytes are of interest and the data is wanted quickly. The use of the ASAP probe is great for making multiple measurements in an extremely quick manner, such as seconds to complete a spectrum for a single insertion. This technique can be used throughout the field of chemistry and more.

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