Determination of vanillin in red and sugar maple sap for the high school classroom

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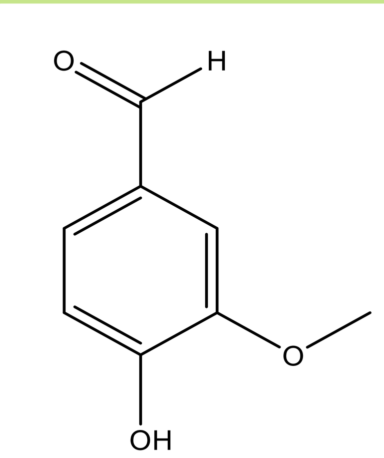
Abstract

This project provided hands on experience with instrumentation and techniques related to high performance liquid chromatography (HPLC). This experience will provide an opportunity to introduce high school students to how HPLC can be used with real world applications. The project uses maple sap and vanillin in order to make the investigation more relevant for students. A standard calibration plot was constructed from preparing vanillin standards of known concentrations. Depending on the quality of the separation of the compounds in the sap samples, the vanillin concentration can be analyzed. In addition to the evaluation of sugar maple sap samples, red maple sap was also investigated. The vanillin concentration was not quantitated in the 2015 sap samples analyzed. Qualitative evaluation showed the presence of vanillin in both sugar maple and red maple 2015 sap samples. In the fall of 2015, the HPLC instrument will be loaned to Prospect Mountain High School (PMHS) where students will analyze maple sap collected from the PMHS campus. The instrument will be used to enhance the student's experience with the science inquiry process.

Introduction

Maple sap is collected from the sugar maple tree, *Acer saccharum*.[1] The tapping season for the collection of sap typically falls between late February to early April when the trees experience freezing temperatures during the night and thawing during the day. The change in temperature allows for an ample flow of maple sap during the day as well as preservation of the sap at night.[2] The composition of maple sap consists of mostly water, sucrose, phenolic compounds such as vanillin, and other minor components. Vanillin, a common component in sap, is a flavoring agent used in the food and beverage industry to give products the characteristic vanilla taste. Examples of products that contain vanillin are the following: vanilla extracts, ice cream, candy, and soda.[3] Maple syrup is a common consumer product that is produced by boiling maple sap.[4] In the state of New Hampshire, 112,000 gallons of maple syrup were produced commercially and valued at $6,474,000.[5] Students will develop a better understanding of the importance of maple sap in maple syrup production.



Vanillin

The goal of this project is to introduce an analytical approach called high performance liquid chromatography (HPLC) for the analysis of vanillin in maple sap to high school students. The HPLC instrument is loaned to high schools for short term use by the UNH Leitzel Center. Students will get hands on experience with an instrument not readily accessible in a high school classroom. Students will use technology to analyze and model data using mathematics and qualitative reasoning (HS-PS4-5, HS-PS4-1).[5] By analyzing the data collected, students will develop critical thinking and problem solving skills that can be utilized to determine a solution to a complex problem.[6] Students will develop a broader understanding of the connections between multiple disciplines and scientific concepts. Examples of concepts that can be incorporated into this project are electromagnetic radiation (HS-PS4-4) and the determination of concentration of solutions.

These disciplinary core ideas and cross cutting concepts coincide with the Next Generation Science Standards (NGSS).[6] This project allows students to collect maple sap that is used to conduct an authentic higher level scientific analysis for vanillin and provide a real world application of how science is applied outside the classroom.

Methods

Reagents

- Vanillin, 99% Fluka Analytical. Certified reference material
- Store Brand 91% (v/v) Isopropyl Alcohol
- Acetic acid 17.5M, glacial; J. T. Baker Inc
- Deionized water
- Maple sap samples taken from PMHS over the 2015 tapping season.

Mobile Phase for HPLC

- Mobile Phase A: aqueous acetic acid (0.06% (v/v) acetic acid)
- Mobile Phase B: aqueous isopropyl rubbing alcohol (20% (v/v) isopropyl rubbing alcohol)

Equipment

- Agilent 1100 series HPLC system with HPG1365B Multiple Wavelength Detector HPG1311A Quaternary Pump HPG1379A Mobile Phase Degasser
- Column: Waters C8 Symmetry (3.9 x 150 mm, 5 µm)
- Rheodyne 7125 injector with a 20 µL injection loop
- 100 µL Hamilton syringe 705 SNR
- 3 mL BD Syringe with Luer Lok tip
- Pall Life Science Acrodisc LC 13 mm syringe filter, 0.45 µm PVDF membrane

Multi-Wavelength Detector Settings

- Signal A: 280 nm, 16 nm bandwidth
- Reference 360 nm, 13 nm bandwidth
- Slit Setting: 4 nm
- Peak width >0.1 min (2s)

Preparation of Standard Solutions

0.1 mg/mL vanillin stock preparation

A mass of 0.010 (±0.001)g of 99% vanillin was placed into a 100 mL volumetric flask containing 25 mL of 91% isopropyl alcohol. The flask was then filled to the 100 mL line with isopropyl alcohol and shaken gently.

6% (w/v) sucrose solution in deionized water preparation

A mass of 30.000 (±0.01)g of sucrose was placed into a 500 mL volumetric flask containing 100 mL of DI water. The flask was then filled to the 500 mL line with DI water and agitated until the sucrose dissolved completely.

10, 25, 50, 100, 150 ng/mL vanillin standard preparation

To make the 25 ng/mL vanillin standard, 16 mL of 91% isopropyl alcohol were placed into a 100 mL volumetric flask. To the volumetric flask, 25 µL of 0.1 mg/mL vanillin stock were added and filled to the 100 mL line with 6% (w/v) sucrose solution. The solution was shaken gently. The 10 ng/mL vanillin standard was prepared by adding 10 mL of the 25 ng/mL vanillin standard to a 25 mL volumetric flask and brought to the 25 mL fill line with 6% (w/v) sucrose solution. The solution was shaken gently.

8 mL of 91% isopropyl alcohol were placed into a 50 mL volumetric flask. To prepare the 50, 100 and 150 ng/mL vanillin standards the appropriate volume of 0.1 mg/mL vanillin stock solution was added to the flasks (50 ng/mL: 25 µL, 100 ng/mL: 50 µL, 150 ng/mL: 75 µL). The 6% (w/v) sucrose solution was then added to the 50 mL fill line and the solutions were shaken gently.

Calibration Plot

Constructed by running the 10, 25, 50, 100, 150 ng/mL standards isocratically (60% mobile phase A, 40% mobile phase B) at a flow rate of 1.00 mL/min. Each standard solution was run in duplicate.

Maple sap analysis

The maple sap samples were thawed to room temperature and then filtered using a Pall Life Science Acrodisc LC 13mm syringe filter containing a 0.45 µm PVDF membrane using a 3 mL BD syringe. The samples were run at a flow rate of 1.00 mL/min isocratically from 0- 9.50 min (60% mobile phase A, 40% mobile phase B). Prior to injecting the next sap sample, the column was flushed for three minutes with the high strength mobile phase (100% B) and then reequilibrated with the mobile phase used for the analysis (60% mobile phase A; 40% mobile phase B) for ten minutes.

Results

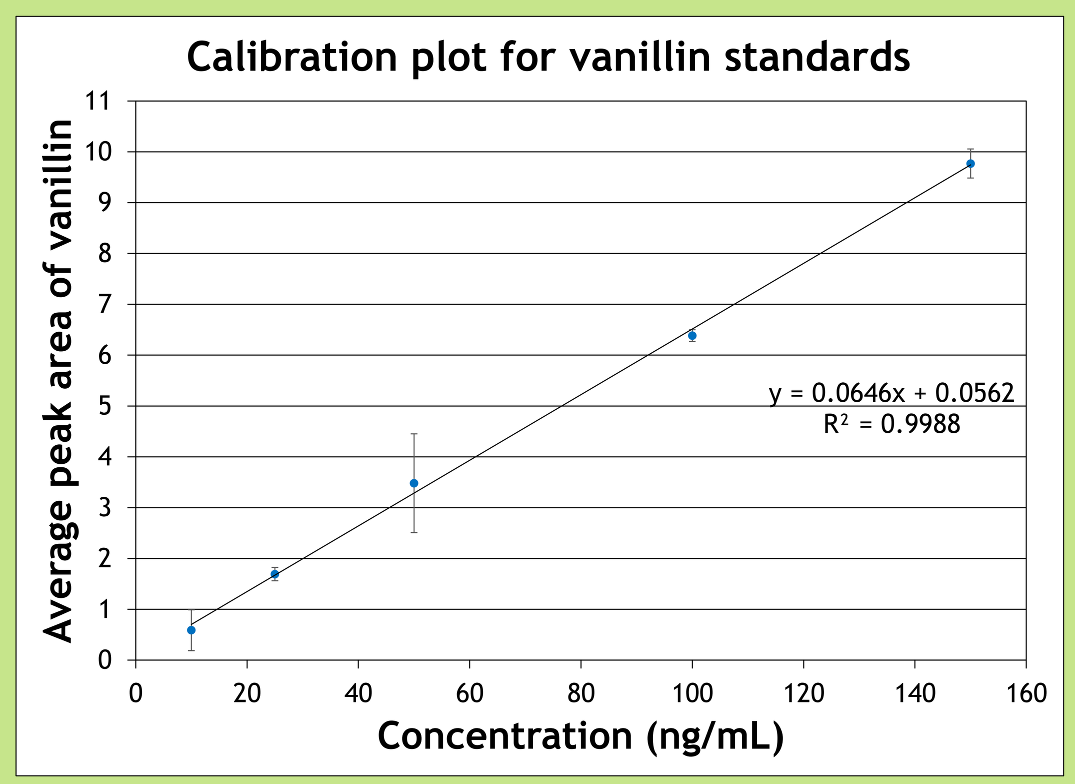


Figure 1: A calibration plot was created from vanillin standards at concentrations of 10, 25, 50, 100, 150 ng/mL. The response with increasing vanillin concentration observed is represented by the calibration plot that can be used to determine the concentrations of vanillin in maple sap samples.

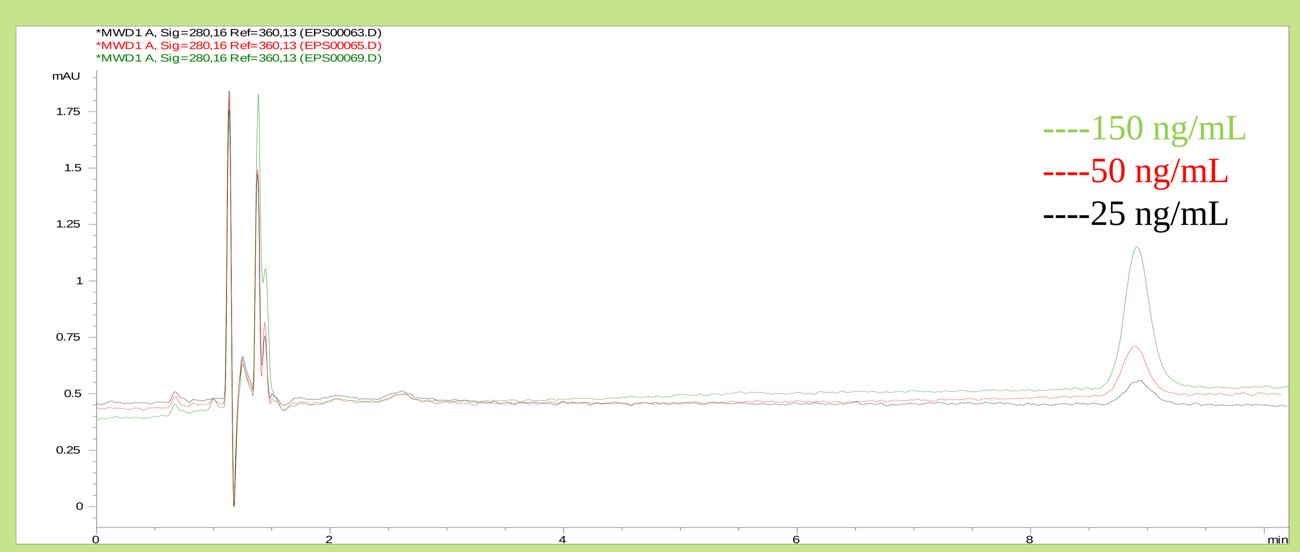


Figure 2: The figure above, is an overlay of the chromatograms of a 25, 50, and 150 ng/mL vanillin standard. At approximately, 8.9 minutes, the vanillin peaks are present. The relative size of the peak increases as the concentration of vanillin in the solution increases.

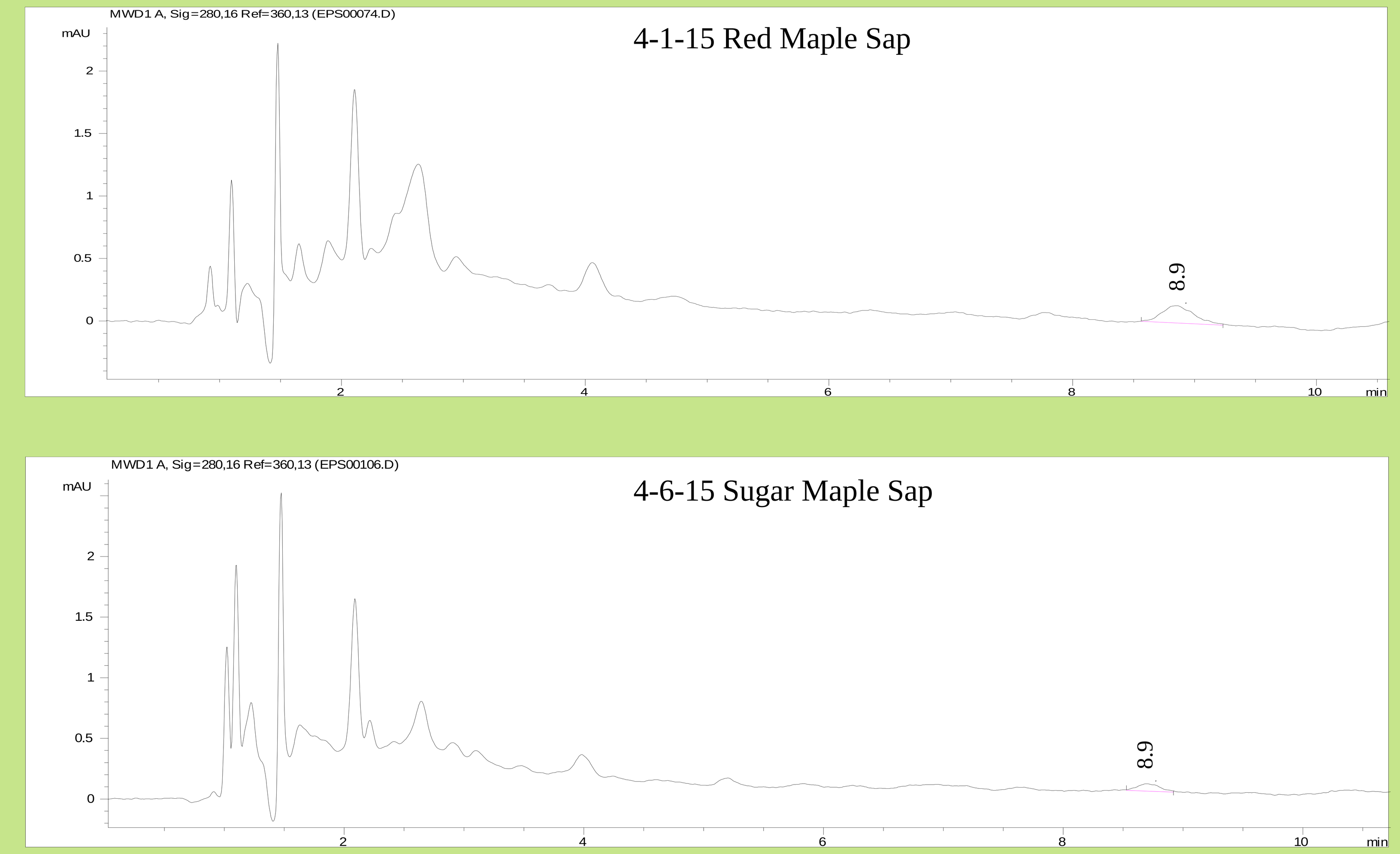


Figure 3: In the figure above, two chromatograms of maple sap samples collected from a sugar maple tree and red maple tree from Prospect Mountain High School on April 1st and April 6th, 2015. In the red maple sap sample, vanillin is present at a retention time of 8.9 minutes. In the sugar maple sap sample, a vanillin peak is also present at 8.9 minutes.

Conclusion

A calibration plot was created from prepared vanillin standards. This plot demonstrates the relationship between the vanillin concentration and the response to be expected for the maple sap samples. Additional investigation and possible modification of the experimental conditions will be required prior to quantitating the vanillin content in the 2015 sap samples collected. However, qualitative variations in vanillin concentrations were observed for sap collected during the 2015 tapping season for both red maple and sugar maple trees.

Future Work

It will be necessary to incorporate aspects of this project in a high school classroom. Features of this project will be modified to fit within the time constraints of a typical high school day. Analysis could include a qualitative or quantitative aspect to view how vanillin concentration changes in a given tree throughout the tapping season and if variations in the vanillin concentration are present in red maple and sugar maple sap samples. It is anticipated in the Fall of 2015 to incorporate this project into a high school classroom. This will be the next step in determining how this project will proceed.

References

[1] Brady, Elizabeth. 2014. Evaluation of Phenolic Compounds in Sap from New Hampshire Sugar Maple Trees by Liquid Chromatography/Mass Spectrometry. Dissertation. University of New Hampshire, Durham, NH.

[2] Carlson, Martha. 2013. Monitoring the health of sugar maple, *Acer saccharum.* Dissertation. University of New Hampshire, Durham, NH.

[3] Good Guide Inc. http://www.goodguide.com/ingredients/37797-vanillin-ingredient-information-reviews (accessed August 1, 2015)

[4] Thériault, M. Antioxidant, antiradical, and antimutagenic activities of phenolic compounds present in maple products. *Food Chemistry*, **2006**, 98, 490-501.

[5] United State Department of Agriculture National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics_by_State/New_England_includes/Publications/Crop_Production/NE%20Maple%20Syrup%20Production.pdf (accessed July 31, 2015)

[6] Next Generation Science Standards For States, By States. **2013**, 1, 258-260.

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