Developing a high school laboratory experiment to measure the vanillin in maple sap

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Abstract

The purpose of this research experience was to gain hands-on experience using high-performance liquid chromatography (HPLC) to analyze the concentration of vanillin in maple tree sap samples. The concentrations of vanillin in sap samples were determined through the use of a calibration curve constructed from known concentrations of vanillin created from a stock solution. It was observed that it is possible to detect the changes of vanillin concentration in maple tree sap over a tapping season. The vanillin concentration in the sap samples peaked 16 days after the tree was tapped at 39ng/mL. After day 16, a decrease in vanillin concentration was observed as the tapping season progressed. The final goal of this research is to implement HPLC instrumentation and analytical procedures into high school chemistry classrooms. This experience would allow students to gain hands-on research experience while giving them the opportunity to collect their own sap samples from the community in which they live. While it was possible to determine the concentration of vanillin, the overall method will need to be optimized so it can be easily integrated into high school chemistry classrooms.

Introduction

Every spring across New England sugar maple trees (*Acer saccharum*) are tapped to collect sap that is used in the production of maple syrup. The process of collecting maple sap was first developed by the indigenous people of North America and later adopted and refined by European settlers[1]. Maple syrup production has grown into an impressive agricultural industry with the US production valued at 132 million dollars in 2013[2]. Maple sap is composed of many different components one of the most important is vanillin, a phenolic aldehyde that is a large contributor to the overall taste of maple syrup. Through the implementation of high-performance liquid chromatography (HPLC), it is possible to analyze vanillin standards for the concentration of vanillin in order to construct a calibration curve. With this calibration curve, the unknown vanillin concentration of maple sap can be determined and monitored by taking multiple sap samples throughout a tapping season. The tapping season typically falls during 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.

One of the long-term goals of this project is to introduce the HPLC analysis of vanillin into high school classrooms around New Hampshire. The UNH Leitzel Center's small and easy to use HPLC instruments will allow teachers to borrow units and use them in their classrooms to analyze maple sap samples collected from the local community by students. An HPLC based educational unit would allow students to gain "real world" experience in performing analytical chemical analysis while gaining a better understanding of the importance of maple sap to maple syrup production, an important agricultural product, occurring within their own community.

Objectives

The objectives of this research investigation were to:

- Gain hands-on experience with HPLC instrumentation and associated laboratory techniques
- Construct and utilize a calibration curve from vanillin standards
- Analyze the vanillin concentration of maple sap samples taken across the 2014 tapping season from a single maple tree
- Improve the overall sample preparation and analysis method to allow for implementation into high school chemistry classrooms

Methods

Reagents

- Vanillin; Fluka Analytical Cat# PHR1245-1G. Certified reference material
- Methanol 0.2µm filtered; HPLC Grade, Fisher Scientific
- Acetic acid 17.5M, glacial; J. T. Baker Inc
- Deionized water
- Maple sap samples taken from the same tree over the 2014 tapping season

Mobile Phase for HPLC

- Mobile Phase A: aqueous acetic acid (0.06% acetic acid)
- Mobile Phase B: 95:5 (v/v) methanol/0.06% aqueous acetic acid
- Mobile Phase C: 50:50 (v/v) methanol/ deionized (DI) water

Equipment

- Agilent 1100 series HPLC system with HPG1365B Multiple Wavelength Detector HPG1311A Quaternary Pump HPG1379A Mobile Phase DEGASSER
- Waters C8 Symmetry (3.9 x 150mm, 5µm)
- Rheodyne 7125 injector with a 20 µL injection loop
- 50µL Hamilton syringe 705 SNR
- 10mL NORM-JECT syringe
- Pall Life Science Acrodisc LC 13mm syringe filter 0.45µm PVDF membrane

Multi-Wavelength Detector Settings

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

Preparation of Standard Solutions

0.1mg/mL vanillin stock preparation

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

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

A mass of 6.00 (±0.01)g of sucrose was placed into a 100mL analytical volumetric flask containing 25mL of DI water. The flask was then filled to the 100mL line with DI water and agitated until the sucrose dissolved completely.

25, 50, 100, 150ng/mL vanillin standards preparation

25mL of 100% HPLC grade methanol were placed into clean 50mL analytical volumetric flasks. Then the appropriate volume of 0.1mg/mL vanillin stock solution was added to the flasks (25ng/mL: 12.5µL, 50ng/mL: 25µL, 100ng/mL: 50µL, 150ng/mL: 75µL). The 6% (w/v) sucrose solution was then added to the 50mL fill line and the solutions were shaken gently.

Calibration curve

Constructed by running the 25, 50, 100, 150ng/mL standards isocratically (82% mobile phase A, 18% mobile phase B) at a flow rate of 1.00mL/min. Each sample was run in duplicate.

Maple sap analysis

The maple sap samples were thawed to room temperature and filtered using a Pall Life Science Acrodisc LC 13mm syringe filter containing a 0.45µm PVDF membrane using a 10mL NORM-JECT syringe. The samples were run at a flow rate of 1.00mL/min isocratically from 0-18.2 min (82% mobile phase A, 18% mobile phase B), and then from 18.3 – 20.3 min (mobile phase A: 40%, mobile phase B: 60%) to wash the column with a higher concentration of mobile phase B, and from 20.3-30.3 min (mobile phase A: 82%, mobile phase B: 18%) to re-equilibrate the column for the next sample.

Results

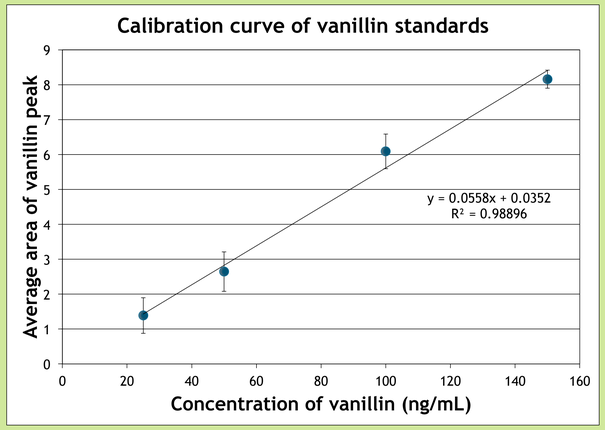

Figure 1: Calibration curve created from the vanillin standards at 25, 50, 100, 150 ng/mL. The incremental increase in vanillin concentration resulted in the creation of a calibration curve that can be used to determine the concentrations of vanillin in maple sap samples.

Days since maple tree tapped	Sample #	Date gathered	Concentration (ng/mL)
0	1	2/23/2014	38
16	4	3/11/2014	39
17	5	3/12/2014	39
20	6	3/15/2014	33
27	7	3/22/2014	*
28	8	3/23/2014	*
39	10	4/03/2014	*

Table 1: This table shows the concentration of vanillin in various sap samples taken throughout the 2014 tapping season. It includes the date the sample was taken as well as the days since the tree was originally tapped. *The concentration of vanillin in samples #7, #8, and #10 were below the range of the calibration curve.

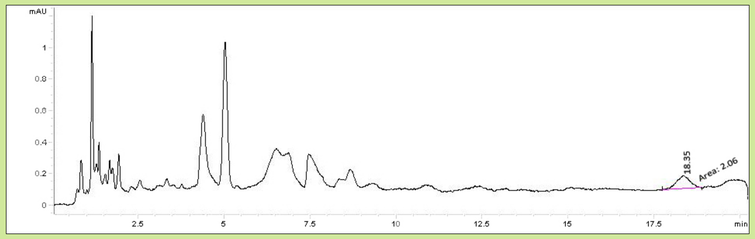

Figure 2: This chromatogram of maple sap sample #4 had a retention time of 18.35 min for vanillin with a peak area of 2.06mAU*s. These values were used in the process of calculating the vanillin concentration in the maple sap sample.

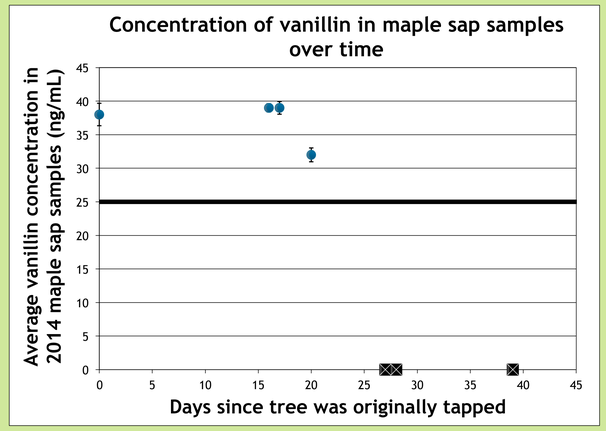

Figure 3: In the figure above, the concentration of vanillin versus number of days since the tree was tapped is shown. *The concentration of vanillin in samples 7, 8, and 10 were below the range of the calibration curve indicated by the black horizontal line at the 25ng/mL level.

Conclusion

It can be concluded that it is possible to create a calibration curve from prepared vanillin standards and utilize the curve to measure the vanillin concentrations of maple sap samples taken over a tapping season. During the 2014 season, the tree reached a maximum vanillin concentration of 39ng/mL 16 days after the tree was tapped. After day 16, a decrease in vanillin concentration was observed as the tapping season progressed.

Future Work

While it was possible to demonstrate that concentrations of vanillin in maple sap samples can be measured using this method, it required many hours of dedicated work. Unfortunately, high school teachers do not have the additional hours to integrate the current method into their classrooms due to their own teaching responsibilities. In the future, sample preparation and analysis methods will need to be optimized to reduce the amount of time required so that teachers can more readily integrate the process into their classrooms.

References

(1) University of Vermont libraries and the agriculture network information center. http://library.uvm.edu/maple/history/ (accessed July 28th, 2014)

(2) United State Department of Agriculture National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics_by_State/New_England_includes/Publications/0605mpl.pdf (accessed July 28th, 2014)

(3) 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.

(4) Carlson, Martha. 2013. Monitoring the health of sugar maple, *Acer saccharum.* Dissertation. University of New Hampshire, Durham, NH.

(5) Deslauriers, Isabelle. 2000. Recovery, Separation and Characterization of Phenolic Compounds and Flavonoids from Maple Products. Thesis. McGill University, Montreal.

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