Abstract
This project provided hands on experience with instrumentation and separation techniques related to high performance liquid chromatography (HPLC). This experience will provide an opportunity to introduce high school students to how HPLC is 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 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 samples were also investigated. Qualitative evaluation showed the presence of vanillin in both sugar maple and red maple sap samples. The vanillin concentration was not quantitated in the 2015 sap samples analyzed. 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 both the sugar maple tree (Acer saccharum) and the red maple tree (Acer rubrum). The tapping season for the collection of sap typically falls between late February to early April when the trees experience freezing temperatures at 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. 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 their characteristic vanilla taste. Examples of products that contain vanillin include maple syrup, vanilla extracts, ice cream, candy, and soda. Maple syrup is a common consumer product that is produced by boiling maple sap. In the state of New Hampshire, 112,000 gallons of maple syrup were produced commercially and valued at $64,764,000. Students will develop a better understanding of the importance of maple sap in maple syrup production.

The goal of this project is to introduce an analytical approach using HPLC for the analysis of vanillin in maple sap to high school students. The HPLC instrument will be loaned to high schools for short term use by the USNH Leitzel Center. Students will get hands on experience with an instrument not readily available in a high school classroom. Also, students will use technology to analyze and model data using mathematics and qualitative reasoning (HS-PS4-5). 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. Students will gain a broader understanding of the connections between multiple content areas and scientific concepts. Examples of concepts that can be incorporated into this project are electromagnetic radiation (HS-PS4-5) and the determination of the concentration of solutions.

These disciplinary over ideas and cross cutting concepts coincide with the Next Generation Science Standards (NGSS). 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
- Generic (90% (v/v) Isopropanol) Alcohol
- Glacial acetic acid, 17.5 M, 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 (0.6%) (v/v) acetic acid
- Mobile Phase B: aqueous isopropanol (20%) (v/v) isopropanol

Equipment
- Agilent 1100 series HPLC system with HPG1345B Multiple Wavelength Detection, HPG1331A Quaternary Pump, and HPG1370A Mobile Phase Degasser
- Columns: Waters C18 Symmetry (3.9 x 150 mm, 5 μm)
- Rhodamine 1215 injector with a 20 μL injection loop
- 100 μL Hamilton syringe-705 SNR
- 3 mL BD Syringe with Luer-Lok™ tip
- Pall Life Science Acrros® LC 13 mm syringe filter, 0.45 μm PTFV membrane

Multi-Wavelength Detector Settings
- Signal A: 200 nm, 16 nm bandwidth
- Reference: 360 nm, 15 nm bandwidth
- Sll Setting: 4 nm
- Peak width: >0.1 min (2s)

Preparation of Standard Solutions
0.1 mg/mL vanillin stock preparation
A mass of 0.005g (100/0.99 g) of 99% vanillin was placed into a 100 mL volumetric flask containing 25 mL of 91% isopropanol 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 mg of sucrose was placed into a 500 mL volumetric flask containing 250 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 μg/mL vanillin standard preparation
To make the 25 μg/mL vanillin standard, 16 mL of 91% isopropanol alcohol were placed into a 100 mL volumetric flask. To the volumetric flask, 25.0 μL of, 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 μg/mL vanillin standard to a 25 μL volumetric flask and brought to the 25 mL fill line with 6% (w/v) sucrose solution. The solution was shaken gently.

To prepare the 50, 100 and 150 μg/mL vanillin standards, 8 mL of 91% isopropanol alcohol were placed into a 50 μL volumetric flask and the appropriate amount of 1 ng/mL, vanillin stock solution was added to the flask (150 ng/mL, 25 μg/mL, 100 μg/mL, 150 μg/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 for vanillin standards
The plot was constructed by running the 10, 25, 50, 100, 150 μg/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 through a Pall Life Science Acrros® LC 13 mm syringe filter containing a 0.45 μm PTFV membrane using a 3 mL BD syringe. The samples were run at a flow rate of 1.00 mL/min isocratically from 0-50 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 re-equilibrated with the mobile phase used for the analysis (60% mobile phase A; 40% mobile phase B) for ten minutes.

Results

Figure 1: The figure above, is an overlay of the chromatograms of a 25, 50, and 150 ng/mL standards.

Average peak area of vanillin

Reference:

Acknowledgements
We would like to thank the following: Sterling Tomlinson, Steve Hale, The USNH Leitzel Center, Elizabeth Brady, Sarah Thomas (PMHS), Walter Shortle, Mike McCrone, Marty McCrone, The John and Kelly Ornell Foundation Fund/Waters Corporation, a USDA Forest Service contract, and the University of New Hampshire. This research was supported with funding from the National Science Foundation’s grant to NH EPSCoR (EPSG-1012425).

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

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. In the Fall of 2015, this project will be incorporated into a high school classroom. This will be the next step in determining how this project will proceed.