Analysis of Phenolic Compounds in New Hampshire Sugar Maple Sap by LC-MS

Elizabeth Brady[1]

Co-authors: Walter C. Shortle[2], Martha Carlson[3], Barrett N. Rock[3], Sterling Tomellini[1]

(1) Department of Chemistry, University of New Hampshire, Durham, NH

(2) USDA Forest Service, Northern Research Station, Durham, NH

(3) Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH

Introduction

Interest in phenolic compounds continues to grow as studies demonstrate a wide variety of their potential biochemical properties through both *in vivo* and *in vitro* experiments.[1] Studies have shown that phenolic compounds possess antimutagenic, antiradical and antioxidant properties that could be beneficial to the prevention of many degenerative diseases.[2] While these compounds may have many interesting impacts on human health, relatively less research is focused on what these compounds mean in terms of the health of plants where phenolic compounds are abundant as secondary metabolites. Our research investigates whether changes in the content or concentrations of certain phenolic compounds in maple sap over the tapping season are indicative of the relative health of the sugar maple trees. We are monitoring 11 selected phenolic compounds using HPLC in samples provided by volunteer maple syrup producers throughout the state of New Hampshire.

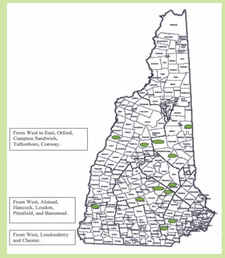

Figure 1. Map of the locations of volunteer producers around the state of New Hampshire.

While methods exist for the detection of phenolic compounds in maple sap,[3] they often require time consuming pre-concentration steps that generate large amounts of chemical waste. We are developing a method for the direct analysis of phenolic compounds in maple sap by HPLC. This direct method may eventually be transferred for use by high school students in an effort to obtain many future years of data. The method poses several challenges including high concentrations of sugar in the sap, working with a biological matrix and achieving the required sensitivity. A method for analysis by LC/MS is also being developed for confirmatory studies.

Goals

- Develop a HPLC method for the direct analysis of phenolic compounds in maple sap
- Develop an efficient LC/MS method to confirm the findings of the HPLC method
- Monitor selected phenolic compounds for changes in the composition/concentration over the 2011, 2012 and 2013 tapping seasons in sugar maple sap from volunteers throughout New Hampshire
- Transfer the chromatographic method to "citizen scientists" as a safe and easy to use HPLC method

Method

Preliminary Studies and HPLC Method Development (Condition A)

Perkin Elmer Binary LC Pump 250 with a 10 µL Rheodyne Injector and a Shimadzu SPD-M20A diode array detector
Column temperature held at 24° C with a column water jacket connected to a PolyScience 9105 Circulating Water Bath
Microsolv HQ C8, 75 x 4.6 mm, 5 µm particle
Mobile Phase A: 0.1% (v/v) TFA in water
Mobile Phase B: 95% (v/v) methanol, 5% (v/v) water
Gradient: 10% B to 52% B over 35 minutes @ 1 mL/min
UV/Vis detection wavelengths at 280 nm and 320 nm

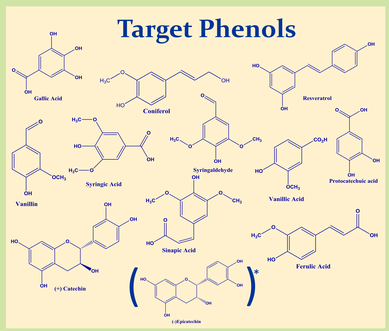

Figure 2. The eleven target phenolic compounds. *(-)-Epicatechin was chosen as a target compound during preliminary studies but later removed due to it being undetected in sap samples.

HPLC Method and LC/MS Conditions (Condition B)

Waters Alliance 2695 LC Pump, Waters 2998 PDA, Waters Acquity SQD LC/MS
Waters Symmetry C8, 3.9 x 150 mm, 5 µm particle
Column temperature held at 24° C with Waters Alliance Column Heater
Mobile Phase A: 0.1% (v/v) formic acid in water
Mobile Phase B: 95% (v/v) methanol, 4.9 % (v/v) water, 0.1% formic acid
Gradient: 18% B to 21% B 0-30 minutes, 21% B to 60% B 30-45 minutes @ 1 mL/min
UV/Vis detection wavelength 280 nm, 10 µL injection

ESCi mode (Combined electrospray and atmospheric pressure chemical ionization)
ES+ and ES- scans from 80-500 m/Z at a cone voltage of 25V and capillary voltage of 3 kV
11 Single Ion Recordings developed , one for each individual phenolic compound

Results

Method Development Challenges
- Safe, easy, inexpensive
- Low concentrations of phenols in maple sap
- Biological matrix
- Sap sucrose content
- Stationary phase lifetime
- Standard solubility issues

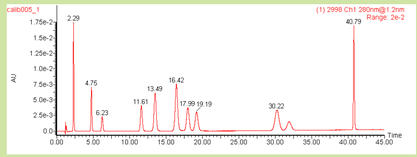

Figure 3. Chromatogram produced by the PDA using condition B for a separation of a solution containing the eleven target phenolic compounds.

Phenolic Compound	Limit of Detection (ng/mL)	Limit of Quantitation (ng/mL)
Gallic Acid	2	5
Protocatechuic Acid	3	9
(+)-Catechin	17	55
Vanillic Acid	3	10
Syringic Acid	5	15
Vanillin	5	14
Coniferol	5	15
Syringaldehyde	4	13
Ferulic Acid	7	23
Sinapic Acid	13	43
Resveratrol	2	4

Table 1. Limits of detection and limits of quantitation in ng/mL.

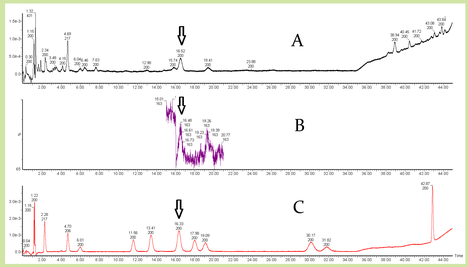

Figure 4. (A) Chromatogram produced by the PDA of a maple sap sample. (B) SIR of the 153 m/z ion for identification of vanillin. (C) Chromatogram produced by the PDA of the 11 phenolic standards.

Figure 4 shows an example of the identification of vanillin in a maple sap sample based on retention time, UV/Vis spectra and the presence of the ion for the programmed Selected Ion Recording (SIR). While vanillin was identified in most samples with relative ease, other phenolic compound identifications in sap proved to be more challenging. Figure 5 displays a (+)catechin standard compared to a potential identification in a sap sample. Two peaks with similar retention times and UV/Vis spectra were present in the sap sample. In addition, the 291 m/z ion corresponding to (+)catechin was present at varying retention times in the area of these signals in different samples. Further investigation is needed to confirm the presence of (+)catechin in the maple sap samples.

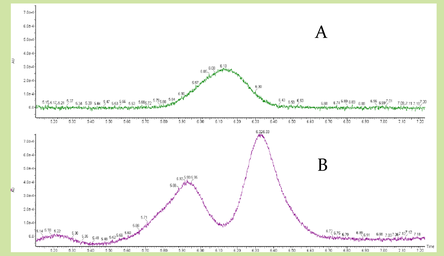

Figure 5. (A) Chromatogram produced by the PDA of a (+)catechin standard. (B) Chromatogram produced by the PDA of a maple sap sample.

Figure 6. Comparison of yearly averages of vanillin from 2011-2013 for 4 maple sap collection sites. The stars indicate collection sites with significant differences in the yearly averages as indicated by the results analysis of variance (ANOVA) tests performed on the data.

Conclusions

- A method was developed for the direct analysis of phenolic compounds in maple sap
- The method successfully separated 11 selected phenolic compounds and achieved sufficient sensitivity with UV/Vis and MS detection to observe the phenolic compounds in maple sap (Fig. 3)
- Five of the eleven selected phenols were identified in the sugar maple sap samples, three require further investigation and three were not identified
- Quantitative data were analyzed over individual tapping seasons and compared between years for both single tree sites and sites with pooled sap samples (Fig. 6)
- Phenolic compounds showed a range of variability throughout tapping seasons
- Mean concentrations compared between 2011, 2012 and 2013 for the same site showed significant differences are present at some collection locations

Future Work

- Identify several prominent unknown peaks
- Shorten analysis time/choose fewer or more select targets
- Improve resolution of specific phenolic compounds
- Analyze phenol content in other parts of the tree
- Analyze sap of other species of trees
- Continue to obtain several years of future data to better assess trends
- Control sample collection methods and frequency
- Compare trees of different ages, diameters and heights
- Analyze off-season sap

We thank the The John and Kelly Ornell Instrumentation Fund/Waters Corporation, a USDA Forestry Service Contract and the University of New Hampshire for instrumentation and financial support.

References

[1] S. Quideau et al. Plant Polyphenols: Chemical Properties, Biological Activities and Synthesis. *Agnew. Chem. Int. Ed.*, **2011**, 50, 586-621.

[2] J. Dai and R. Mumper. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*, **2010**, 15, 7313-7352.

[3] Kermasha, S.; Goetghebeur, M.; Dumont, J. Determination of Phenolic Compound Profiles in Maple Products by High-Performance Liquid Chromatography. *J. Agric. Food Chem.*, **1995**, *43* (3), 708-716.