



# Identification of Elderberry (*S. nigra*) using HPTLC, HPLC and UV-Vis and Detection of its Adulterants

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11/15/2021 – AM Session





# Intro



Brittany Brodziski  
QC Analytical Supervisor

## Primary Focus

- ID (HPTLC, FTIR, DNA)
- ICP Minerals/Heavy Metals
- HPLC Potency

## Fun Facts

- Have been with Nature's Way for 12 years
- Favorite HPTLC chromatogram – guggul resin
- I have a cat named Beaker
- Scuba certified
- Devoted DIY'er

# Nature's Way

*Trust the Leaf*



Headquarters in Green Bay,  
WI



Manufacturer of dietary  
supplements and vitamins



Industry participant for over  
50 years



QC Lab recently received ISO  
17025:2017 accreditation





## Elderberry aka European Elder (*Sambucus nigra*)

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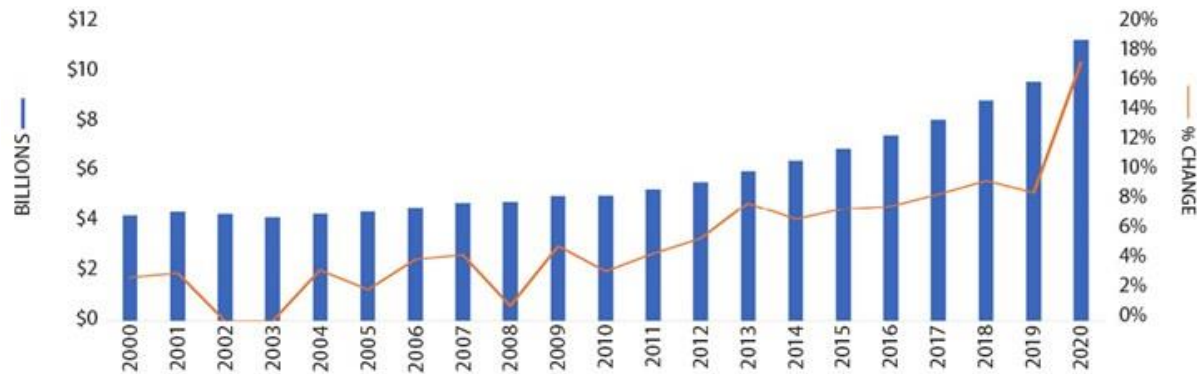
- Native to Europe
- “Complete medical chest”
- Traditionally used as a remedy for immune support
  - Syrups
  - Tinctures
- Rich in Vitamin A and C
- High anthocyanin content
  - 7% and 14% anthocyanin extracts common in marketplace





# Cause for Adulteration

- High consumer demand
  - Strong focus on immune, stress relief



US Retail Sales of Herbal Supplements

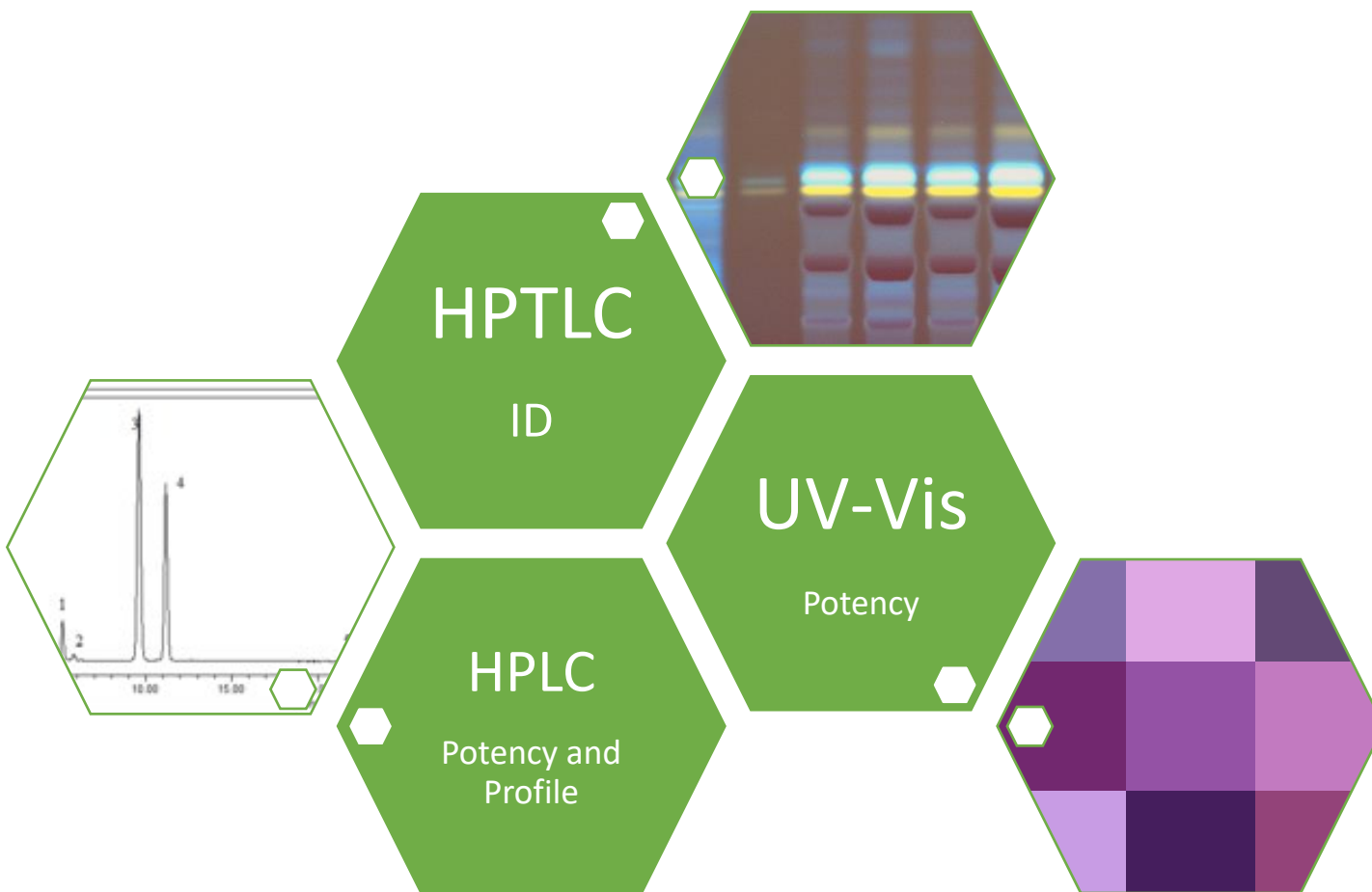
Table 4. Top-Selling Herbal Supplements in 2020 — US Mainstream Multi-Outlet Channel				
Rank	Primary Ingredient	Latin Binomial	Total Sales	% Change from 2019
1	Elder berry	<i>Sambucus nigra</i> and <i>S. canadensis</i>	\$275,544,691	150.3%

Source: HerbalGram 2021; Issue 131, pp. 52.65

- Supply chain issues
  - MFG closures, transportation, customs
- Demand > Supply



# Orthogonal Approach



# Anthocyanin Content – UV-Vis

## Advantages

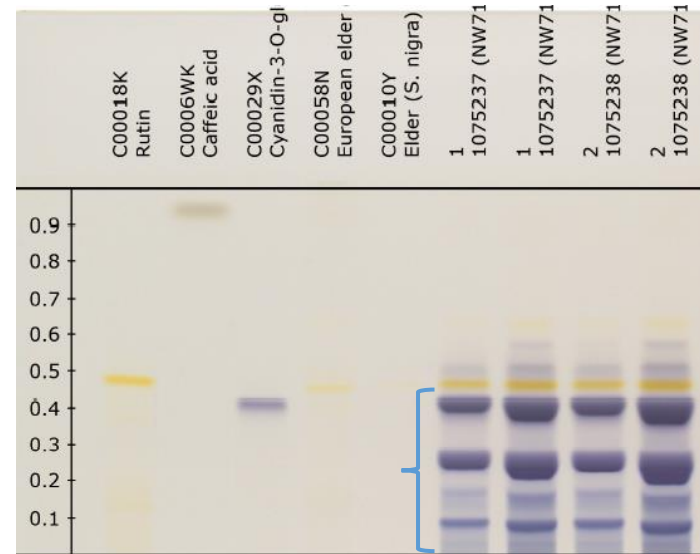
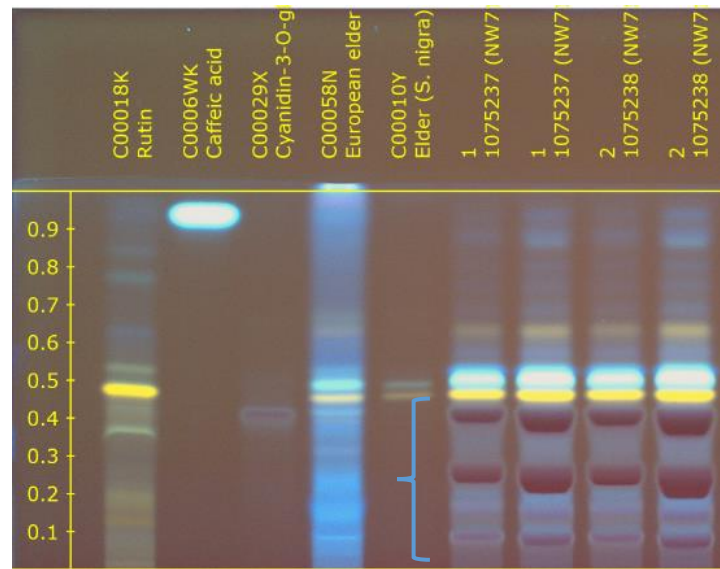
- Easy to use
- Common laboratory equipment

## Disadvantages

- Unable to detect adulteration
- Measures absorbance of all anthocyanin-like compounds at the specific wavelength

- AOAC: "Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method"
- *"Rapid and simple spectrophotometric method based on the anthocyanin structural transformation that occurs with a change in pH (colored at pH 1.0 and colorless at pH 4.5)."*

# Identification by HPTLC



MP: EtAoc:HoA:HoF:H<sub>2</sub>O (100:11:11:27)

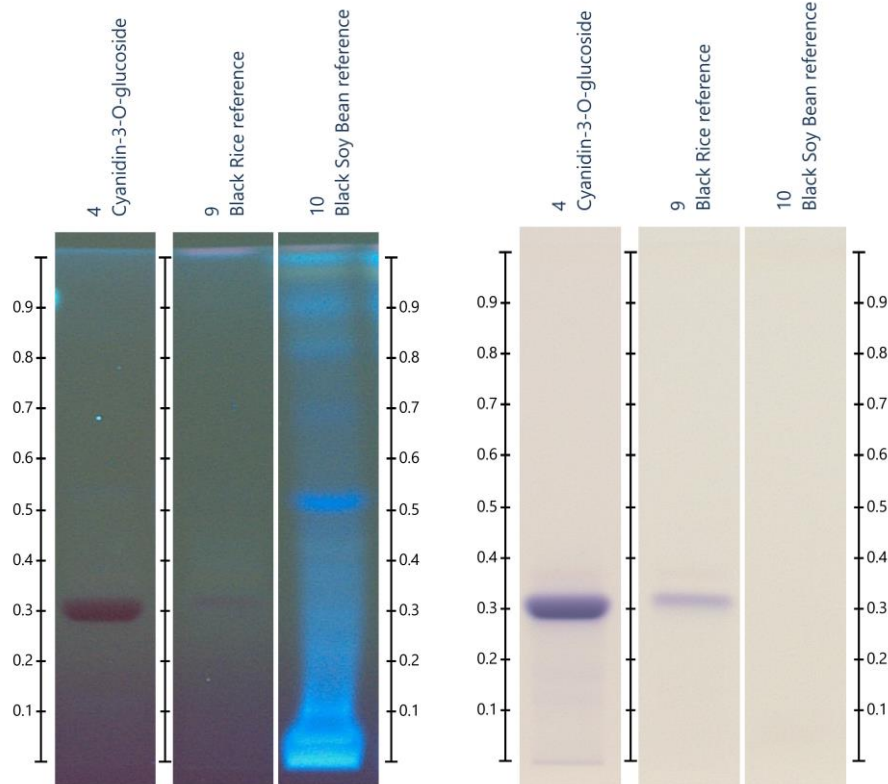
DR: NP Reagent

- *S. nigra* fruit shows a yellow and blue zone corresponding to rutin and chlorogenic acid, respectively (orange bracket). Multiple blue or purple zones below rutin are also present (cyanidin derivatives) (blue brackets).
- Common Adulteration Characteristics
  - Additional, prominent zones present in the upper third of the chromatogram.
  - Lacking cyanidin derivatives (purple zones) below rutin.



# HPTLC Disadvantages

- Spiking difficult to detect
- Some known adulterants contain same anthocyanins
- Or... adulterant chromatogram is not ideal using current method

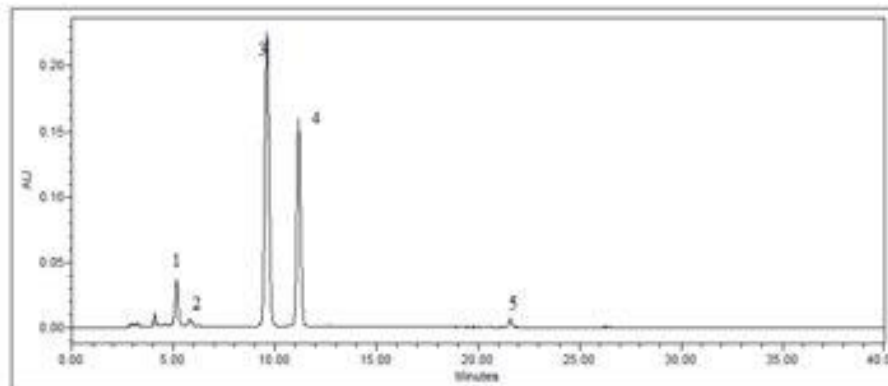


# HPLC-UV Anthocyanin Content

- "European Elder Berry Dry Extract is prepared from the quick-frozen ripened fruits of *Sambucus nigra* L. (Family Adoxaceae/Viburnaceae) by extraction with alcohol. It contains NLT 17% of total anthocyanosides calculated as the sum of the chloride salts of cyanidin-3-O-sambubioside-5-O-glucoside, cyanidin-3,5-di-O-glucoside, cyanidin-3-O-sambubioside, and cyanidin-3-O-glucoside on the anhydrous basis; and NMT 0.2% of cyanidin as chloride salt on the anhydrous basis."

## USP European Elder Berry Dry Extract RS

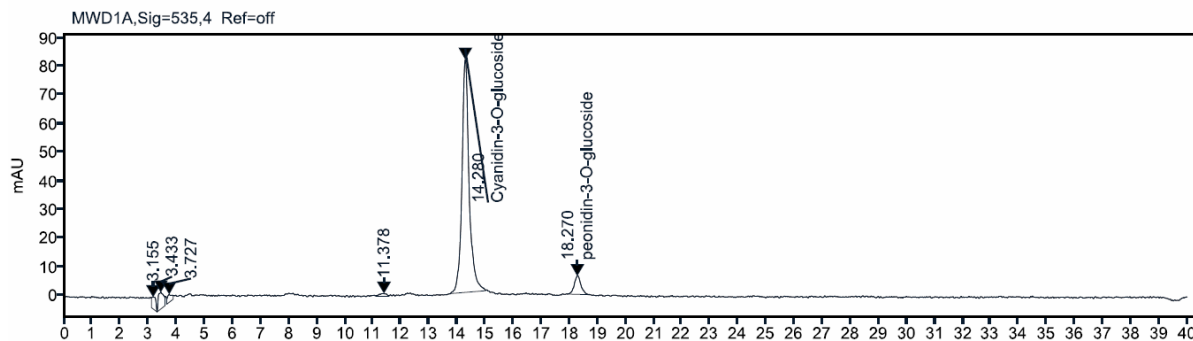
<b>Catalog Number:</b> 1234340
<b>Lot:</b> F076M0
<b>Monograph:</b> European Elder Berry Dry Extract
<b>Publication:</b> PF 43(2)
<b>Test:</b> Content of Anthocyanosides and Cyanidin
<b>Sample:</b> Standard Solution C



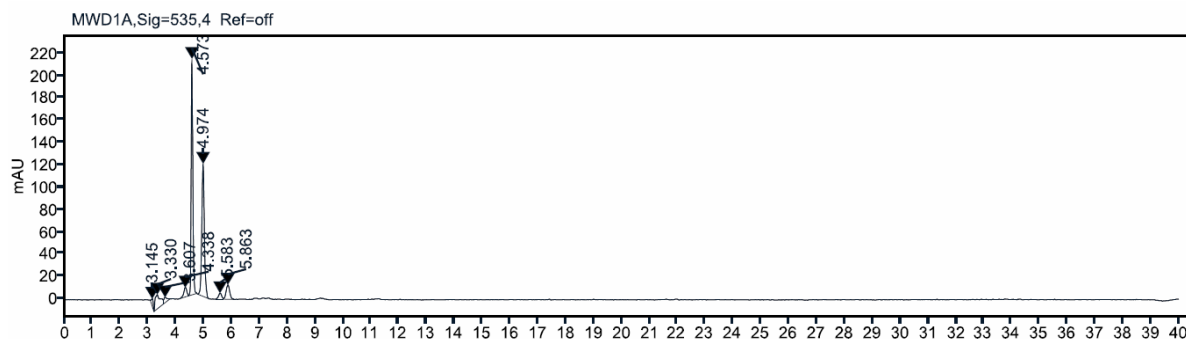
1. Cyanidin-3-O-sambubioside-5-O-glucoside
2. Cyanidin-3,5-di-O-glucoside
3. Cyanidin-3-O-sambubioside
4. Cyanidin-3-O-glucoside
5. Cyanidin

# Potential Adulterant HPLC Profiles

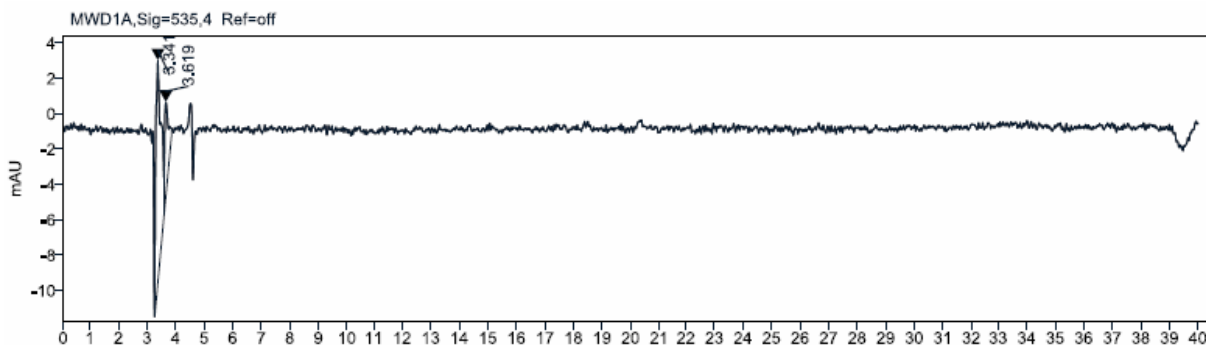
Black rice



Beet root



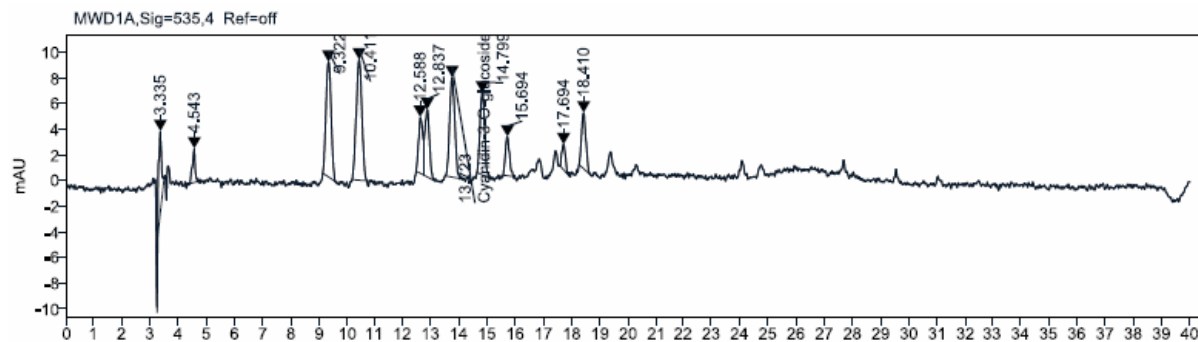
Blueberry fruit



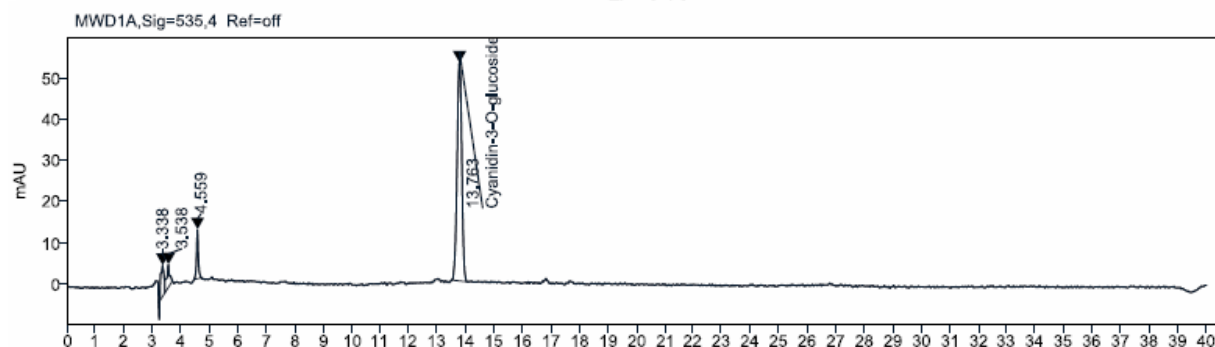


# Potential Adulterant HPLC Profiles

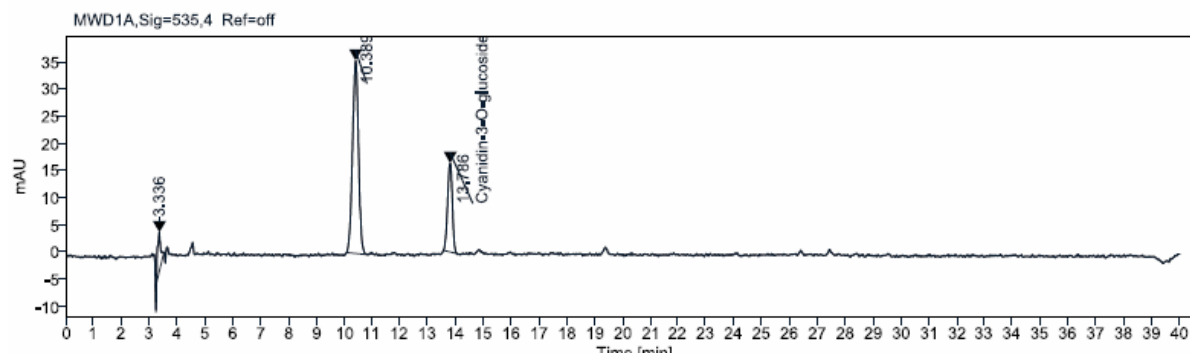
Bilberry



Acai

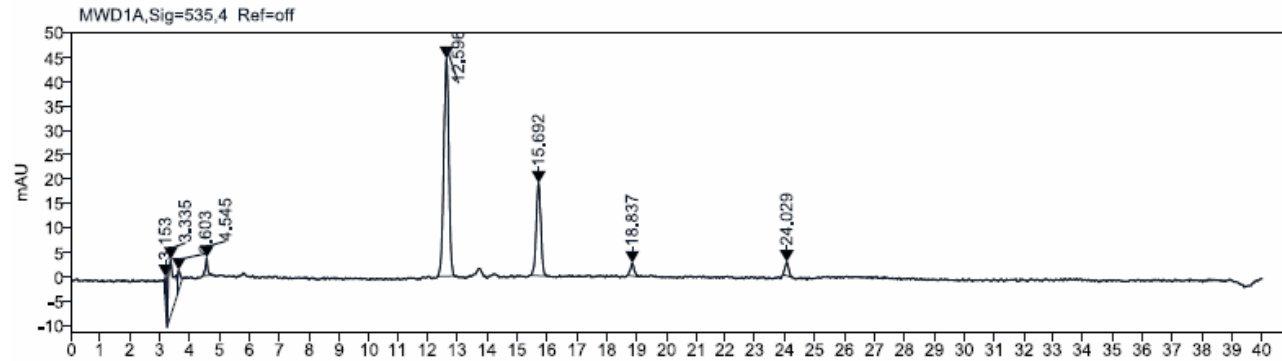


Black Currant

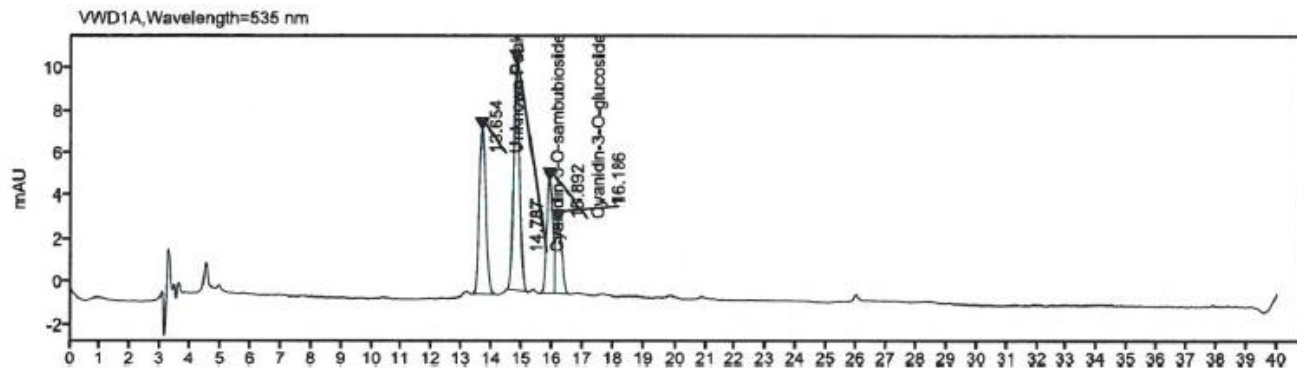


# Potential Adulterant HPLC Profiles

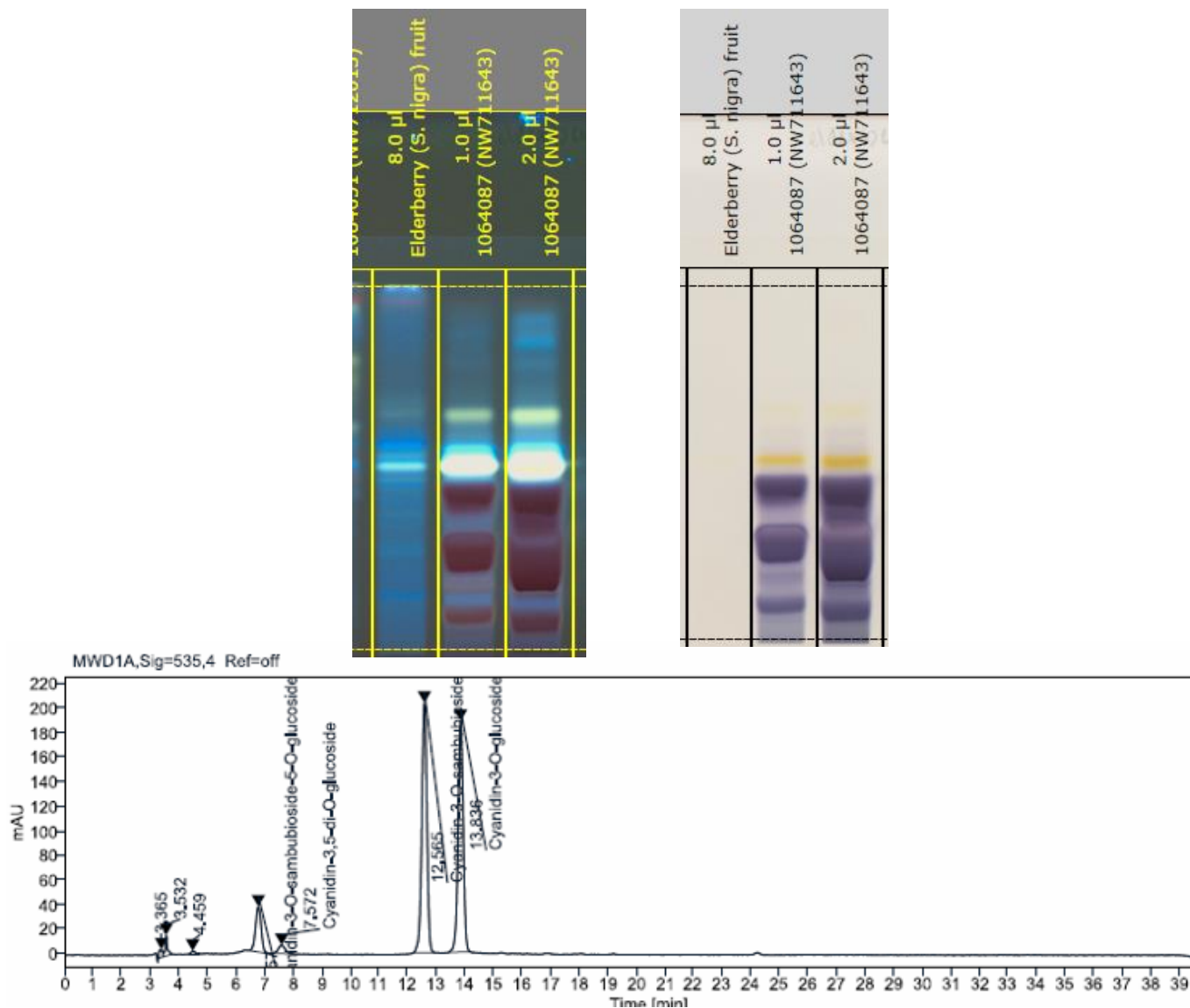
Aronia berry



*Sambucus  
cerulea*  
fruit



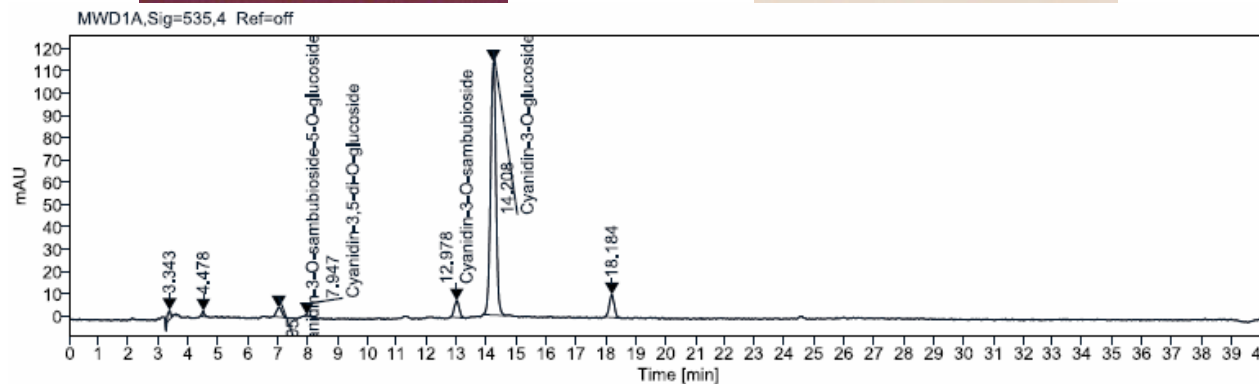
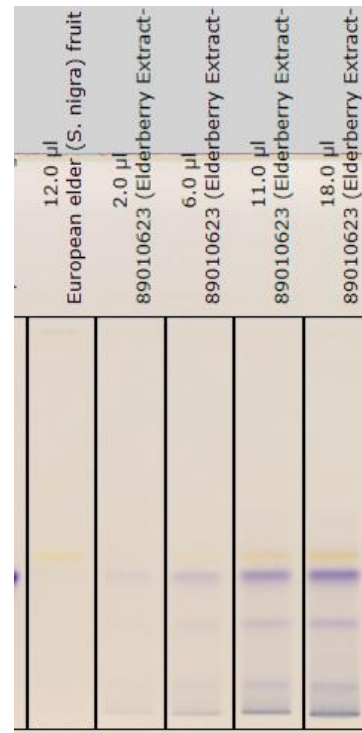
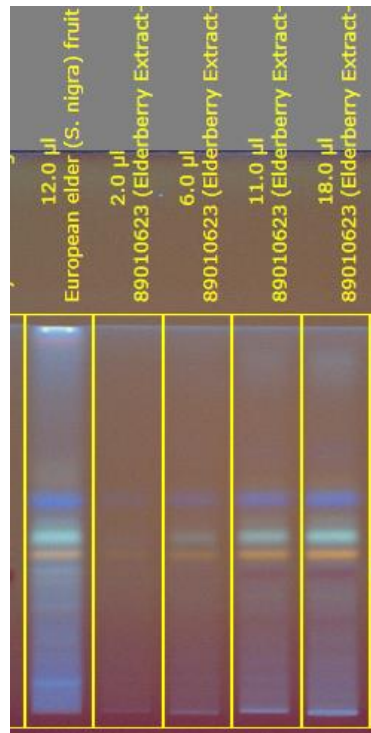
Conforms to *Sambucus nigra* fruit, 14% SE



UV-Vis: 17.5%; HPLC: 17.0%; Cyanidin: <0.2%



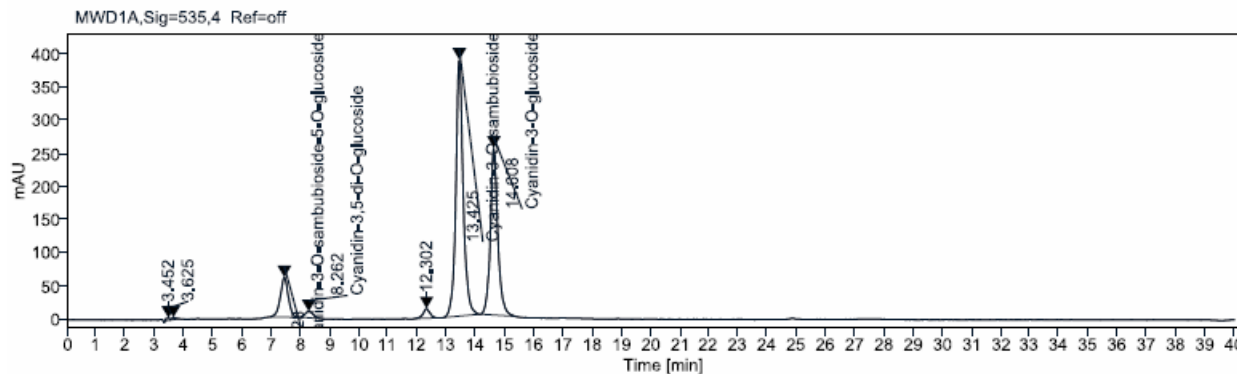
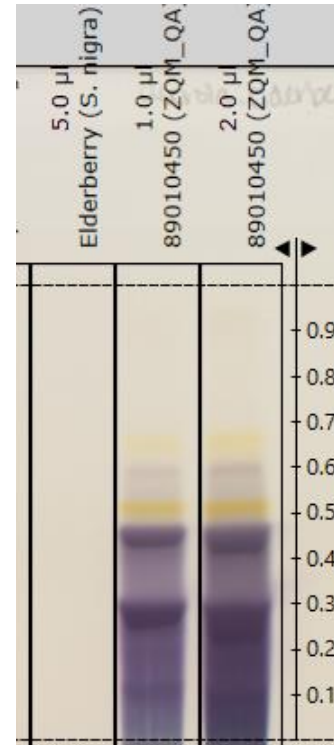
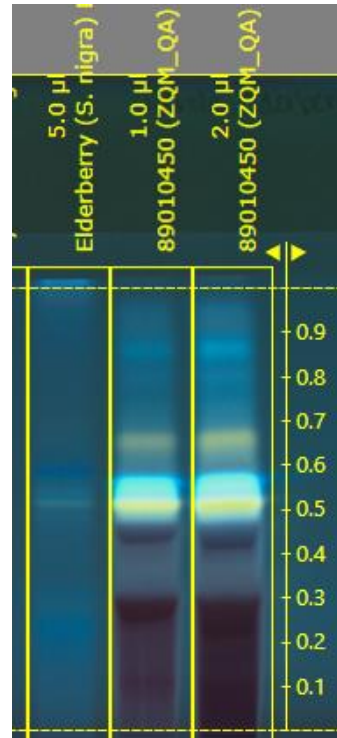
Conforms to *Sambucus nigra* fruit, <14% SE



UV-Vis: 0.20%; HPLC: 0.148%; Cyanidin: <0.2%

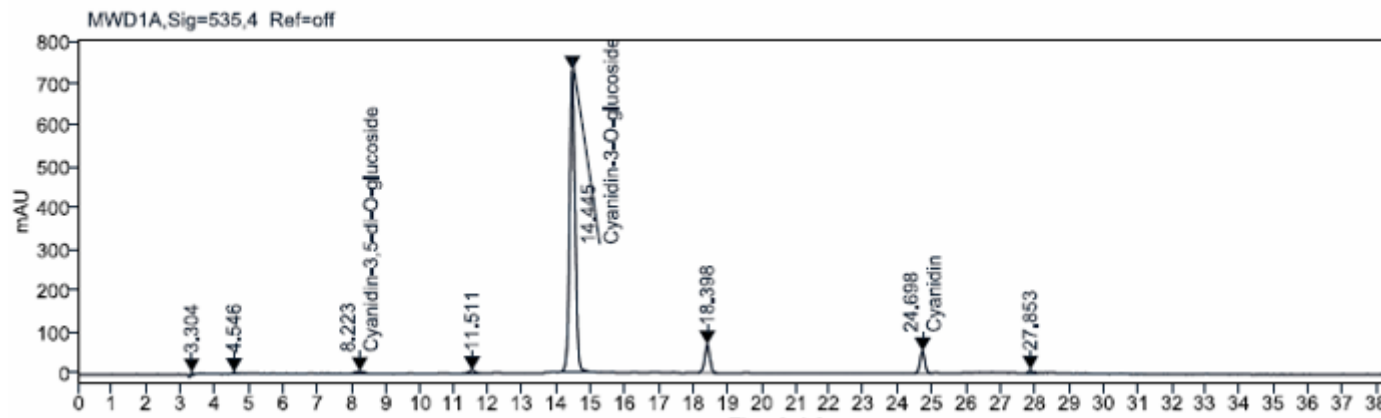
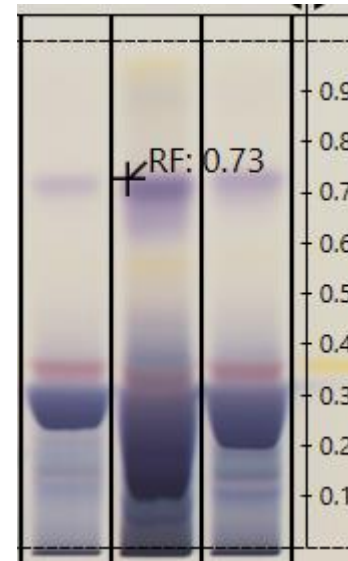
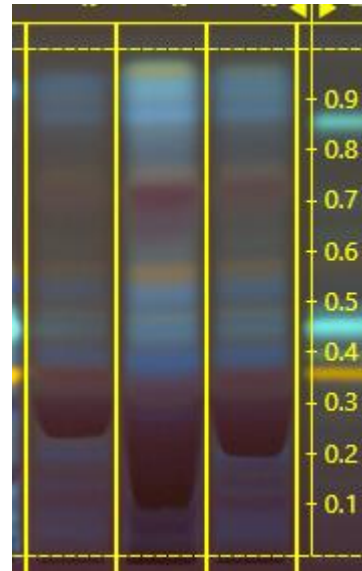


# Indeterminate; *S. nigra* 30% SE



UV-Vis: 32.7%; HPLC: 32.6%; Cyanidin: <0.2%

Meets claim by UV-Vis, Does not conform to *S. nigra*

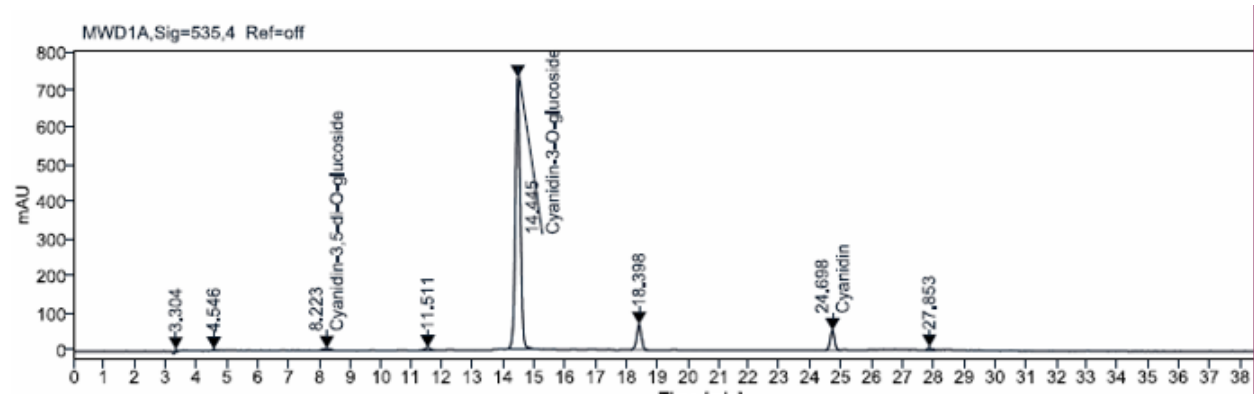


UV-Vis: 16.3%; HPLC: 12.3%; Cyanidin: ?%

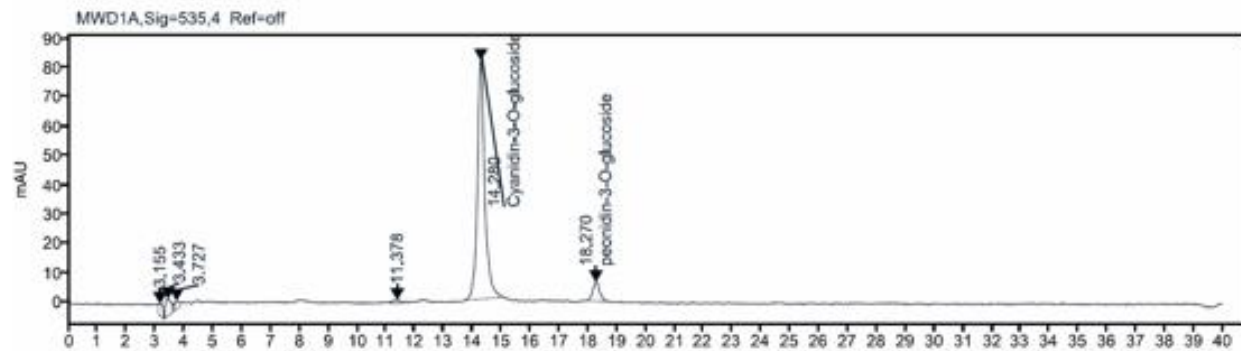


# Could it be?

Sample

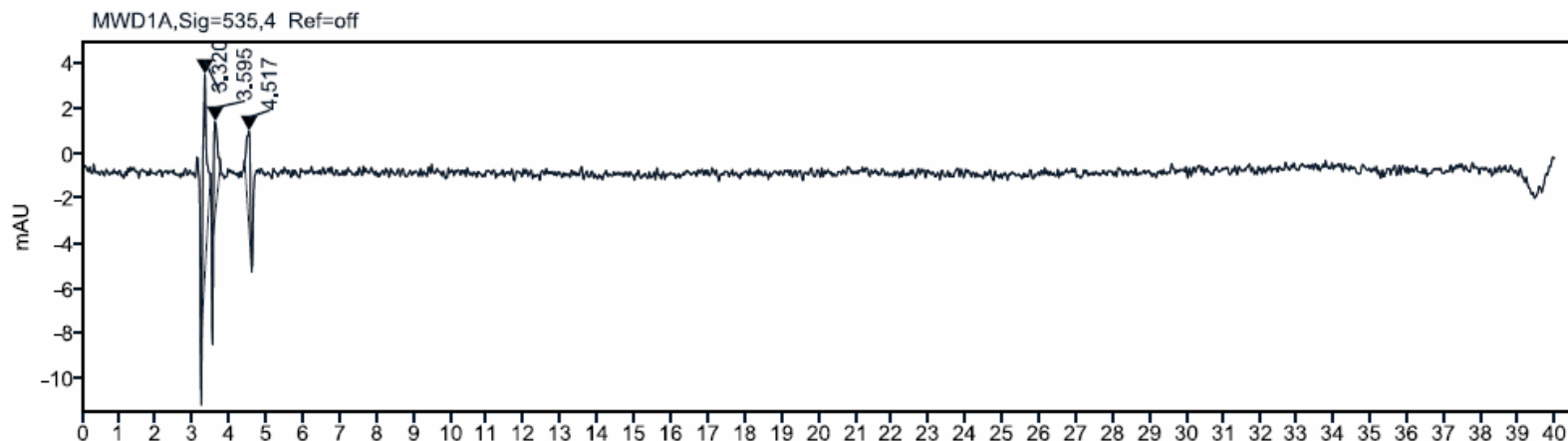
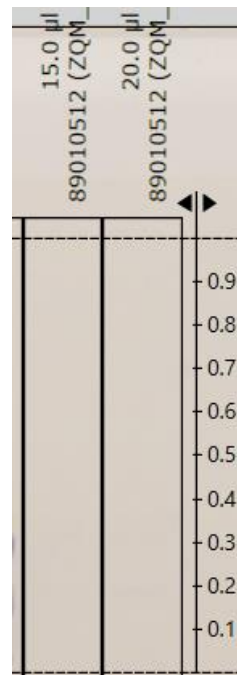
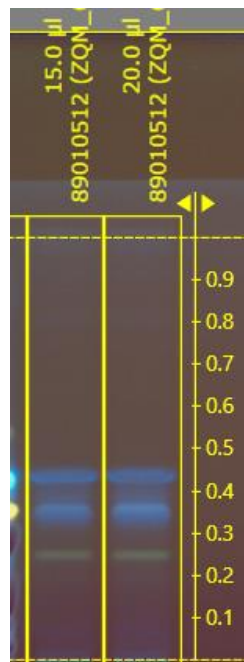


Black rice



UV-Vis: 16.3%; HPLC: 12.3%; Cyanidin: ?%

# Does not conform



UV-Vis: **ND**; HPLC: **ND**

# Results

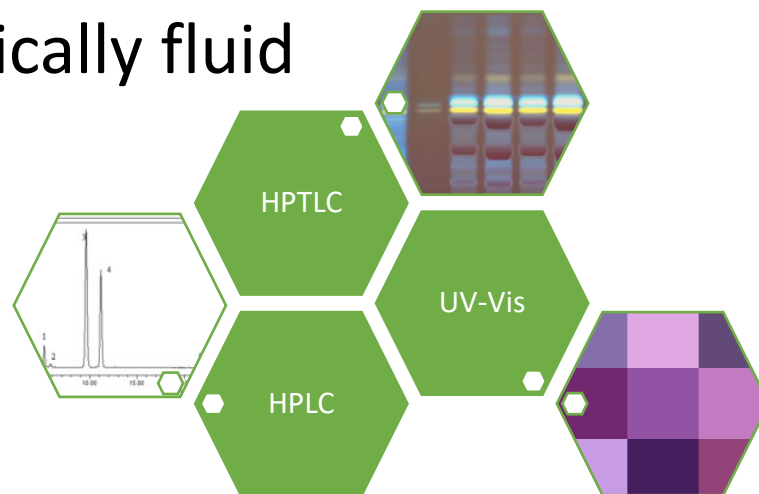
- ~40% of samples showed non-conformance
  - Sourced, bulk elderberry extracts
  - Finished products
- *Tales from the Elder: Adulteration Issues of Elder Berry*, American Botanical Council, HerbalEGram Issue 3, March 2021





# Quality Control

- Market surges, supply shortages drive cause for adulteration
- Remain vigilant and technically fluid



# Questions?

## Contact info

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