

Development of a Method for Identifying and Quantifying Epicatechin in Cinnamon Extract Supplement Capsules

Danielle Valls^{1,2}, Gilbert Castañeda², Traci Carranza², Jeff Dorsett², Mohammad Khalid², David B. Rawlins¹, Brandon Renninger¹, and Casey L. Sayre¹

¹Roseman University of Health Sciences, College of Pharmacy, Henderson, NV

²ProCaps Laboratories, LLC, Henderson, NV



BACKGROUND

- Cinnamon has a long history of use as a medicinal plant.⁶
- Polyphenolic type-A polymers present in cinnamon are believed to have an insulin-potentiating effect, which may be beneficial in diabetes.^{1,2,4,5,7,9}
- Currently, there are no published HPLC methods to identify and quantify type-A polymers in Cinnamon 400™ capsules.
- An analytical method is necessary to evaluate Cinnamon 400™ for its beneficial concentration of type-A polymers.
- Since it is present in the structure of all type-A polymers, epicatechin was selected as the primary analyte.

PURPOSE

- This study will aim to develop an HPLC method that can be used by industry to detect and quantify type-A polymers in Cinnamon 400™

Compound of Interest: Type-A Polymers

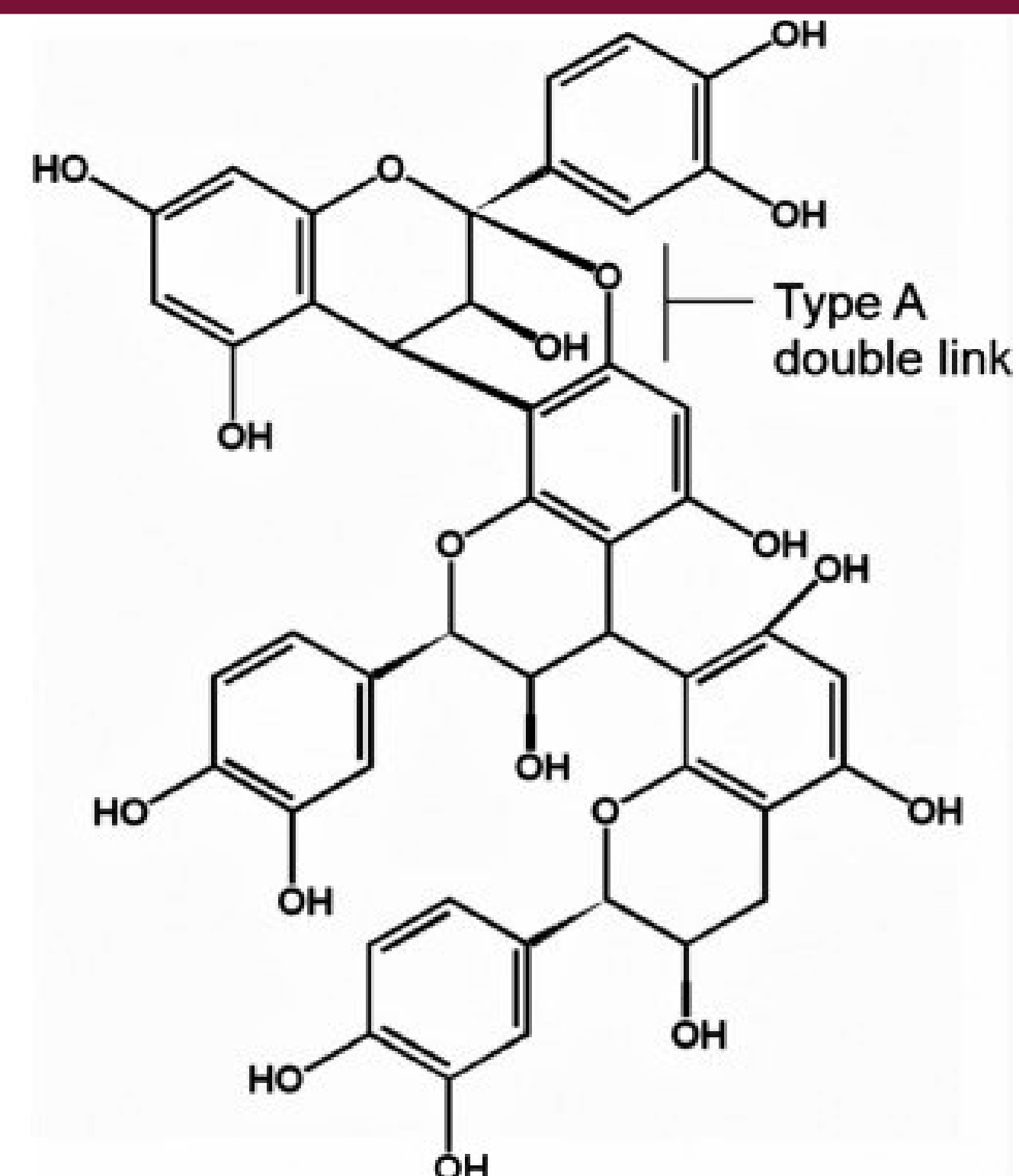


Figure 1. Type-A Polymers: Doubly-linked polymers that have both A and B-type linkages; [A-type]>[B-type]. Molecular weight of 864 Da as a trimer and 1152 Da as a tetramer¹.

Analyte of Interest: Epicatechin

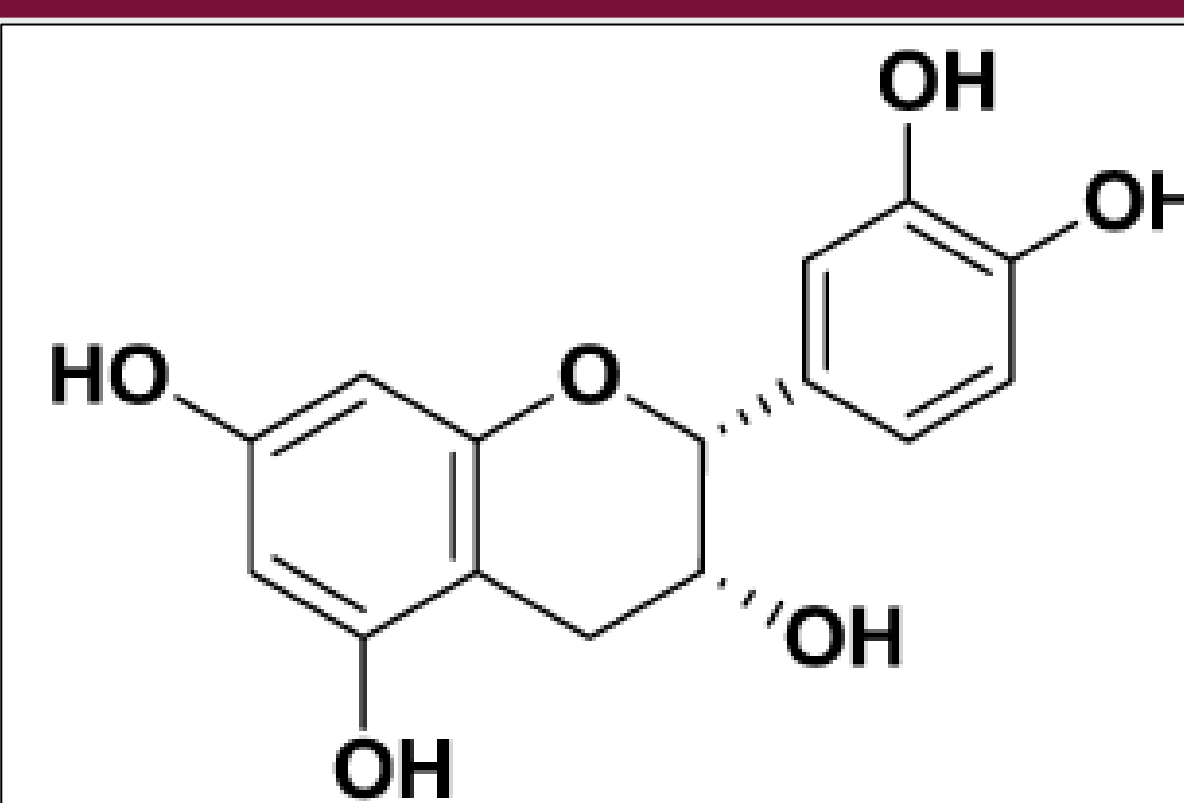
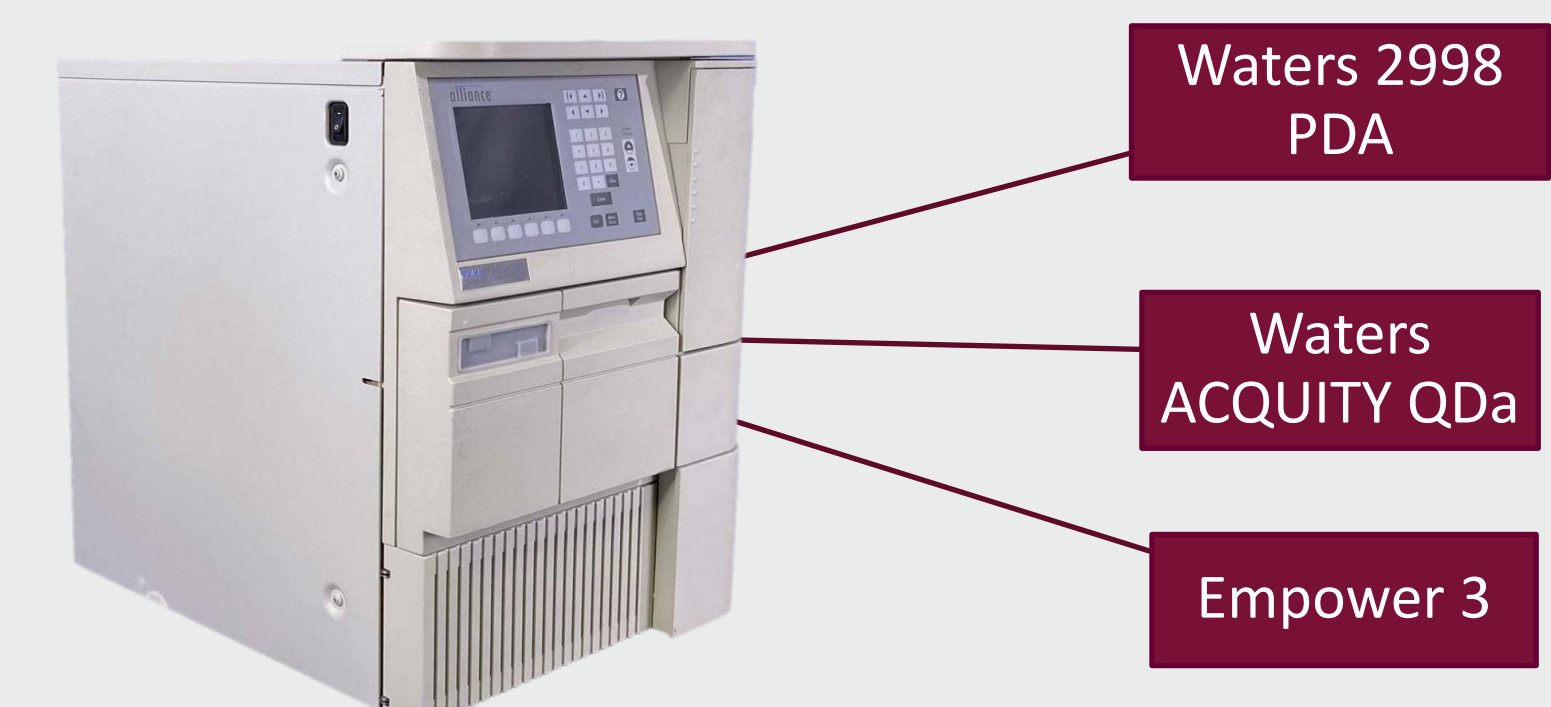


Figure 2. (-) Epicatechin: Exclusive of type-A polymers. Molecular weight of 290.27 Da.^{3,8}

METHODS

Instrumentation and Software



Waters 2998 PDA

Waters ACQUITY QDa

Empower 3

- Concentration:** Photodiode Array (PDA) detector measures light absorption within a broad spectrum of wavelengths
- Mass and Purity Detection:** ACQUITY QDa detector can confirm analyte(s) of interest and purity of standards and samples
- Collecting and Analyzing Data:** Empower 3 software allows for peak detection, integration, and quantification

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METHODS CONTINUED

Preliminary Solubility Study

Sample* (#)	Weight (mg)	Volume (mL)	Solvents (HPLC Grade)	Solvent Ratio	Observations
1	10.5	1mL	ACN	100	Didn't dissolve well
2	12.6	1mL	ACN, Water	50/50	Dissolved well, few particles
3	12	1mL	ACN, Water	25/75	Dissolved better than #2 (Best Ratio)
4	13.6	1mL	ACN, Water, Acetone	35/32.5/32.5	Dissolved OK, still some particles
5	21.5	1mL	Methanol	100	Didn't dissolve well
6	11.7	1.5mL	Water	100	Didn't dissolve better than the #3
7	12.4	1.5mL	Water, Methanol	66.66/33.33	A lot settled on bottom, some dissolved
8	10.2	1mL	Ethanol	100	Similar to ACN alone, didn't dissolve well
9	11.4	1mL	Ethanol, Water	50/50	Dissolved OK, cloudy and particles, dissolved better than #10
10	11.6	1mL	Ethanol, Water	25/75	Dissolved OK, cloudy and particles
11	13.5	1mL	Water	100	Didn't dissolve well, many particles

Table 1. Cinnamon extract solubility data; Acetonitrile (ACN), Deionized Water (Water), Methanol, and Ethanol solubility ratios
*Aqueous cinnamon extract is a raw material used in the Cinnamon 400™ supplement capsules; Donated by ProCaps Laboratories, LLC.

Method Parameters and Conditions for the HPLC

Stationary Phase	Luna® Omega 5µm C18 (250 x 4.6mm)	Gradient		
Mobile Phase(s)	A: 0.1% formic acid in water B: acetonitrile	Time (min)	Flow (mL/min)	Mobile Phase (%)
Wavelength	280 nm	0	1.8	92 / 8
Column Temp.	40°C	20	1.8	75 / 25
Sample	Aqueous cinnamon extract	30	1.8	10 / 90
Reference Standard	Epicatechin ≥90%*	35	1.8	10 / 90
Flow Rate	1.8 mL/minute	37	1.8	92 / 8
Polarity	Negative	45	1.8	92 / 8
Run Time	45 minutes			
Mode	Gradient			
Injection	5µl			

Table 2. Parameters and conditions in reversed phase.^{1,3}
*(-)-Epicatechin ≥90% (CAS: 490-46-0) purchased from Sigma-Aldrich⁸

RESULTS

Epicatechin Calibration Curve

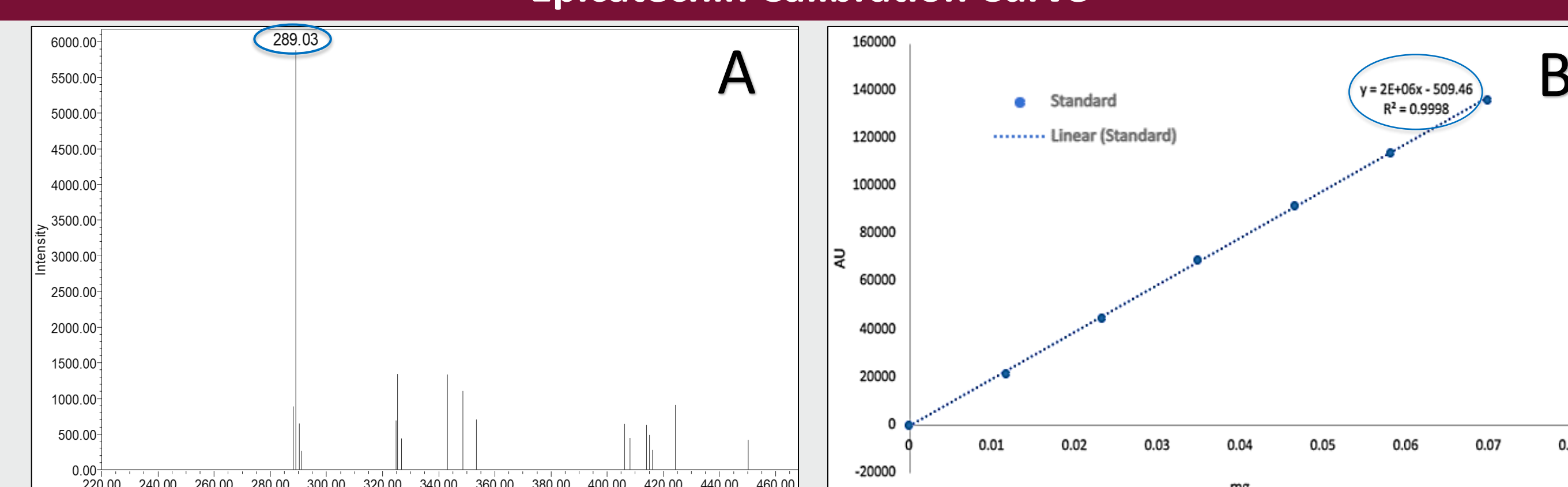


Figure 4. Epicatechin Standard Curve Analysis: (A) Mass Spectrophotometry (MS); All standard samples were confirmed for both analyte presence and purity using the QDa (y-axis: intensity, x-axis: m/z). Target molecular weight for epicatechin during detection and analysis was 289.27 Da (B) PDA Concentration Values; Calibration curve values obtained using PDA detector at 280nm (y-axis: AU, x-axis: mg).

ID	Level	Concentration (mg)	Area (AU)
Blank	1	0.00000	0
S1	2	0.01164	21463.75004
S2	3	0.02328	44721.60014
S3	4	0.03492	68896.13989
S4	5	0.04656	91906.27553
S5	6	0.05820	114078.5004
S6	7	0.06984	136257.4599

Table 4. Epicatechin calibration curve; MW= 289.03 Da, retention time (RT): 7.888 minutes, $r^2 = 0.9998$, $y = 2E+06x - 509.46$

RESULTS CONTINUED

LC-MS Data for Aqueous Cinnamon Extract

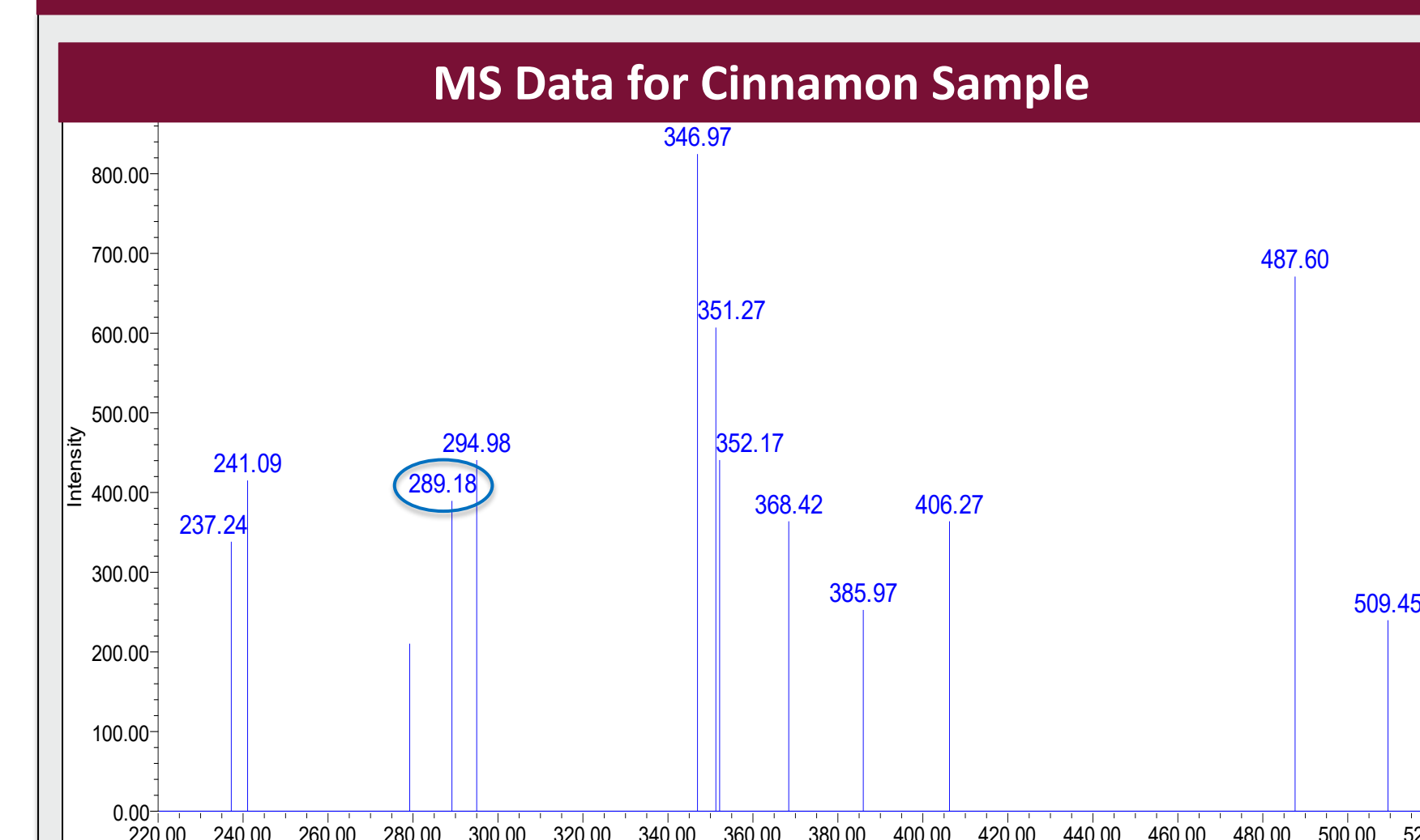


Figure 5. Raw Material MS Confirmation Content Analysis for Epicatechin; QDa data confirmed the presence of Epicatechin in the raw cinnamon sample; mw= 289.18 Da present around 7.670 minutes at 280nm.

HPLC Data for Cinnamon Sample

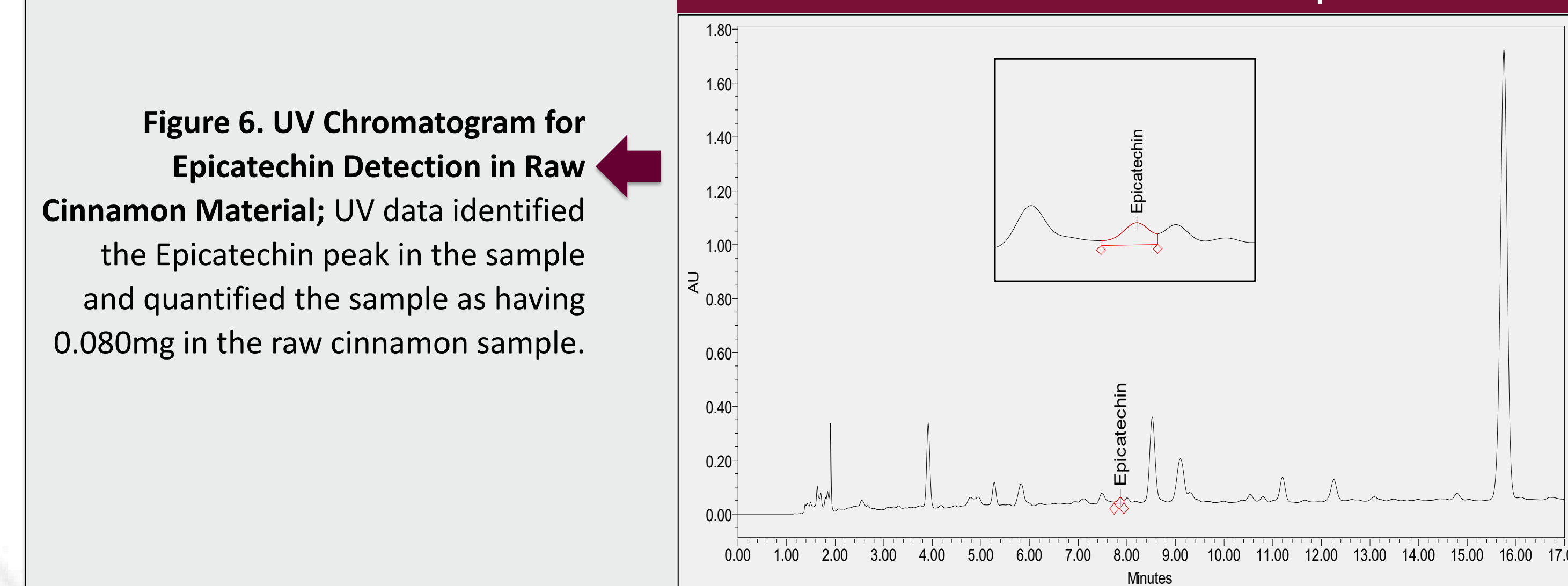


Figure 6. UV Chromatogram for Epicatechin Detection in Raw Cinnamon Material; UV data identified the Epicatechin peak in the sample and quantified the sample as having 0.080mg in the raw cinnamon sample.

Quantification Using Calibration Curve

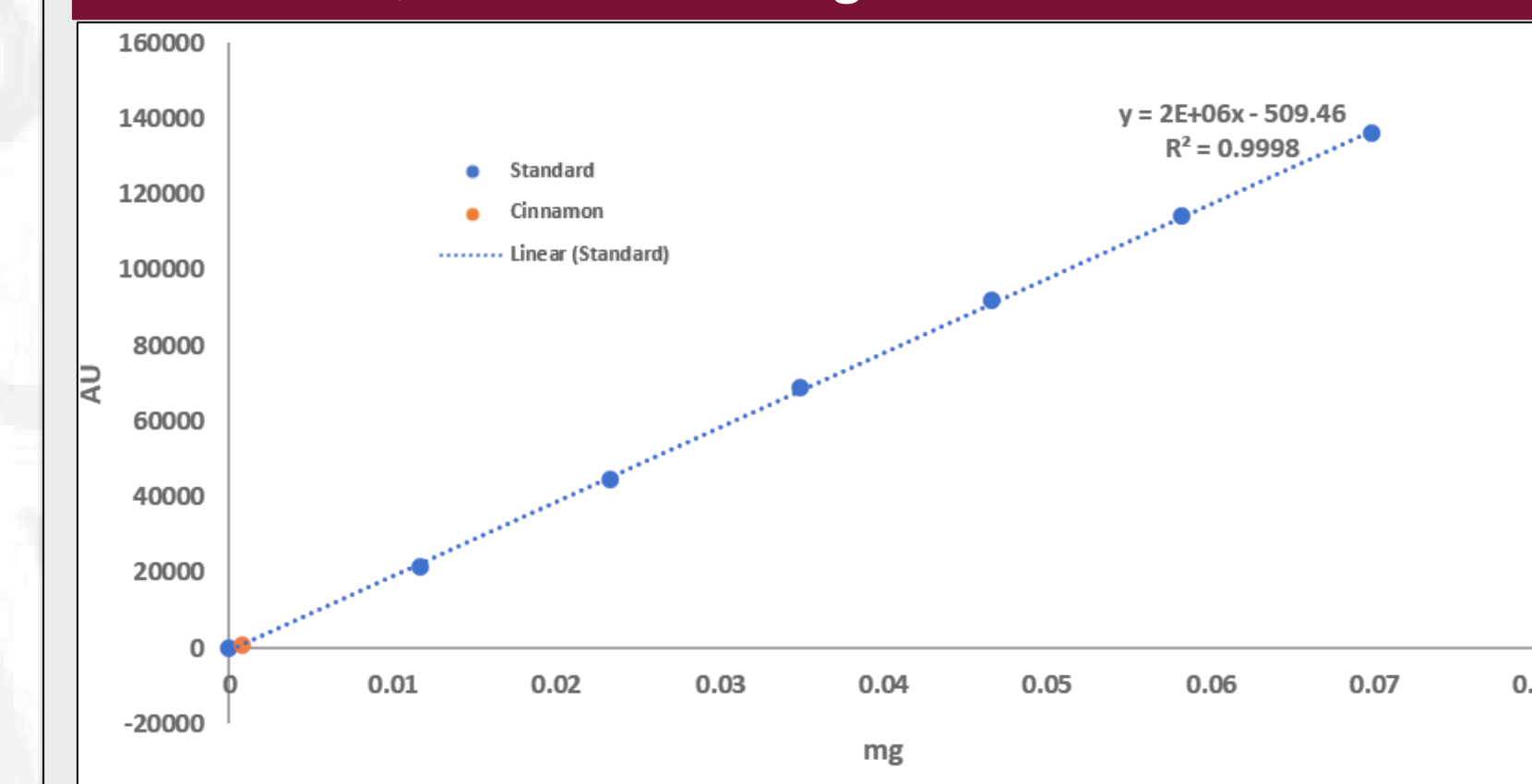


Figure 7. Quantification for Estimation of Type-A Polymers Using Epicatechin; UV absorption quantified the amount of epicatechin in the cinnamon sample at 7.670 minutes to 0.001mg. Low end of calibration curve.

ID	Analyte Detected	RT (min)	Area (AU)	Calc. Concentration (mg)
Cinnamon	Epicatechin	7.670	1034	0.001

Table 5. Estimated concentration of type-A polymers in Cinnamon

FUTURE DIRECTIONS

- HPLC methodology will be verified by using an existing validated method acquired by the Roseman's College of Graduate Studies in collaboration with ProCaps Laboratories.
- Ability to quantify type-A polymers in the finished product for Cinnamon 400™.
- More research is needed to understand the beneficial benefits of type-A polymers in cinnamon.

ACKNOWLEDGMENTS

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