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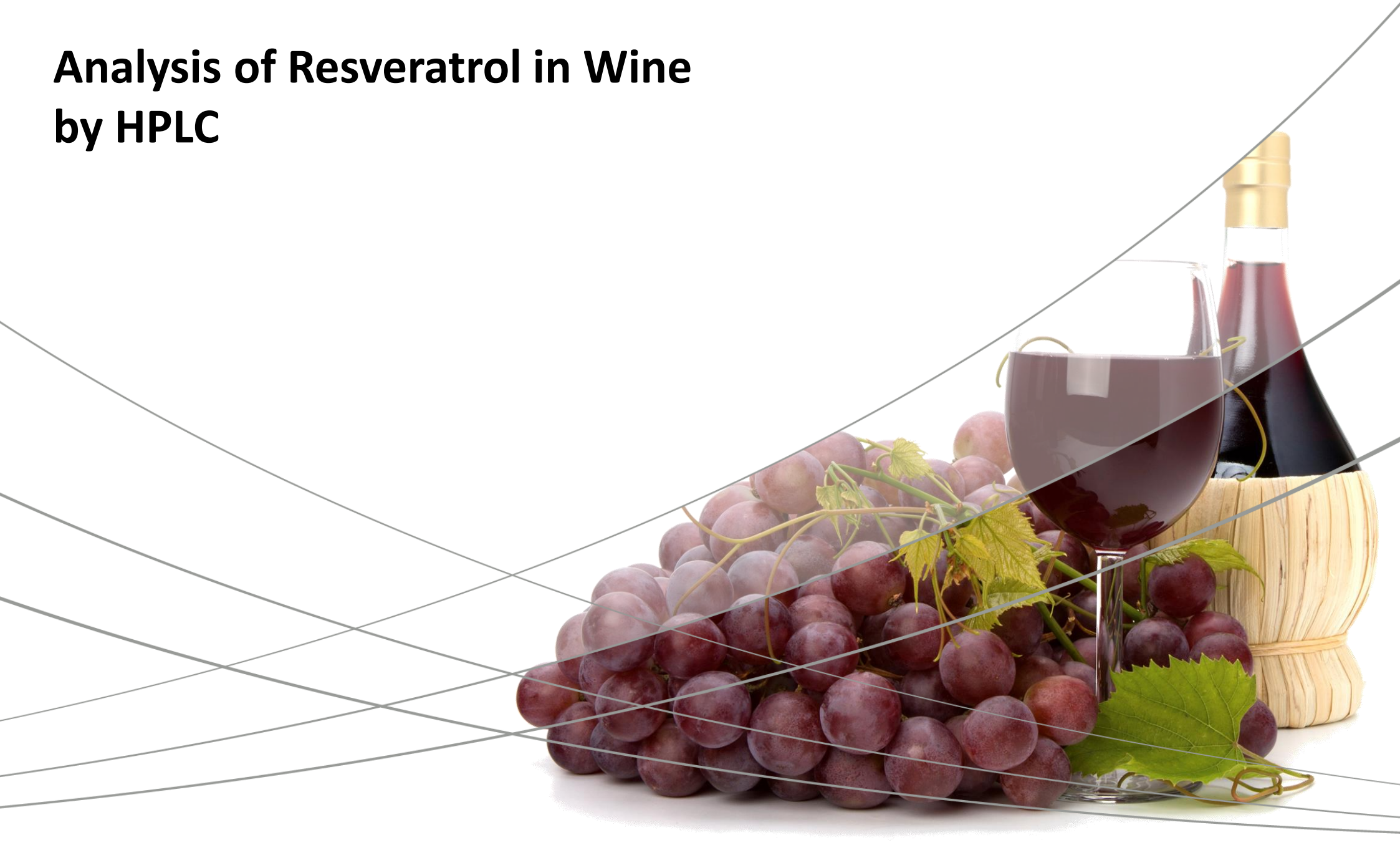
Excellence in Science

Analysis of Resveratrol in Wine by HPLC



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Outline

- Introduction
- Resveratrol
  - Discovery
  - Biosynthesis
- HPLC separation
- Results
- Conclusion

Introduction

- Composition of flavoring, coloring and other characteristic ingredients in wine is largely dependent on make and storage
- White and red wine differ not only in color of the grapes, but also in the way they are produced

- For white wine, grapes are pressed and the resulting must is fermented into an alcoholic beverage “Mostgärung”
- To produce red wine the grapes are squashed to produce a slurry of grape juice, pulp and skin. During fermentation of this mash, the colorants are extracted into solution. Pressing takes place after fermentation “Maischegärung”.

Introduction

- Only fermentation from mash can result in extraction of compounds from grape skin and seeds
- Therefore red wine contains a number of compounds that are not found in white wine.
- Also after fermentation, the process of “aging” during the period of storage in a wooden cask results in distinct changes and a unique profile of over 1000 different compounds in each lot.

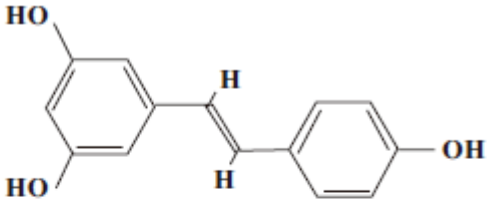
Introduction

Apart from water and ethanol, red wine contains in average:

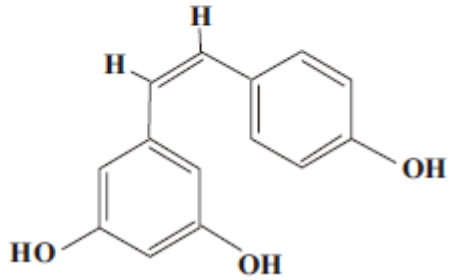
Component	Amount	Charecteristic
Glycerol	1 %	Oily, sweetness
Organic acids	0.4 %	Acidity
Anthocyanins	0.1 %	Red colour
Catechins		Bitterness (> 20 mg/L), antioxidants
Tannins		Astringency, bitterness
Flavonols		antioxidants
Other compounds	0.5 %	

Resveratrol

- Another molecule in red wine, however, has been the main focus of health benefits in recent years: **Resveratrol**



trans - Resveratrol



cis - Resveratrol

- It has been shown in studies that resveratrol, as well as having antioxidant properties, can help prevent high blood pressure (hypertension) in mice and also has anti-inflammatory effects. It's also suspected to have anticancer, and chemopreventive abilities.

Resveratrol - Discovery

- Reseveratrol was first discovered byJapanese researcher Michio Takoaka and his group in 1939.
- They collected **veratrum grandiflorum**, a stout, exotic and flowering plant from the Hokkaido Island.
  A phenolic compound was obtained by crystallization and the molecular formula was identified.
- The new compound was named RES (resorcinol family), VERATR (from veratrum grandiflorum) OL (used to indicate hydroxyl groups)

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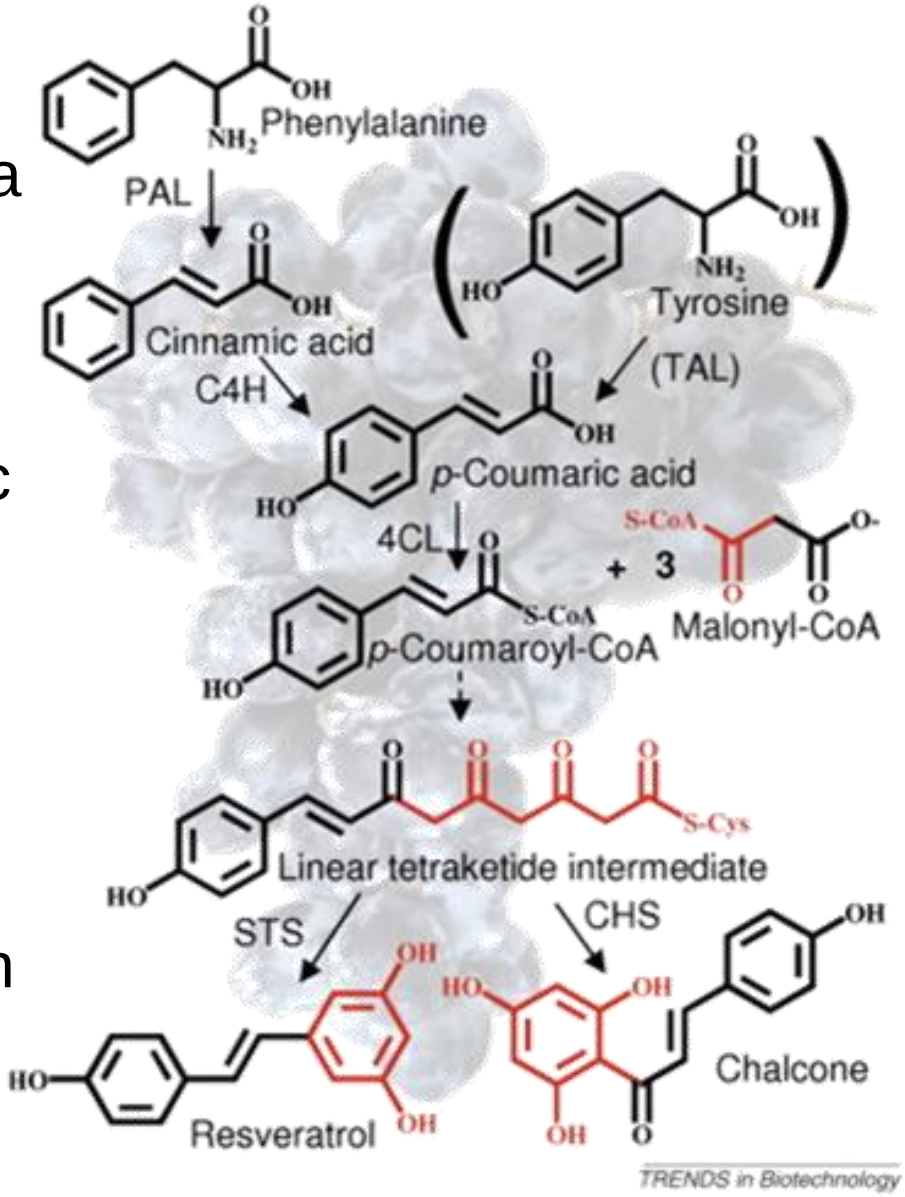
Resveratrol - Biosynthesis

- Resveratrol is found in the skin of red grapes, peanuts, in many berries and a few other plants… but how does it get there?
- Resveratrol is derived from p-coumaric acid which is an intermediate in lignin production. The two key enzymes are Coenzyme A (CoA) Ligase (4CL) and Stilbene Synthase (STS)
- Resveratrol is produced in plants when they are exposed to stress such as UV-light, disease and pests.



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HPLC separation

- Reversed phase HPLC is most appropriate to separate the cis- and trans isomers from each other.
- Fluorescent detection is used for high sensitivity (LOQ ≤ 0.015 mg/L[1] vs. 0.3 mg/L[2] using DAD)
- Wine sample complexity requires gradient optimization to resolve the trans-resveratrol peak from the rest of fluorescent stilbenes and polyphenols.
- Fast analysis using Nexera X2 UHPLC system equipped with PDA and RF-20 AXS Fluorescent detector.

[1] J. Sep. Sci. 2007, 30, 669 – 672
[2] J. Agric. Food Chem., Vol. 51, No. 18, 200

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Method Development

Columnn: GIST C18, 100 x 2.1 mm, 2 µm

Mobile Phase:

A: Water incl. 0.2 % Formic Acid

B: Acetonitrile

GE Program

0 min	BCONC	20%
4 min	BCONC	50%
4.01 min	BCONC	20%

Flow rate: 0.6 ml/min

Temperature: 50 °C

PDA detection: 190 – 360 nm

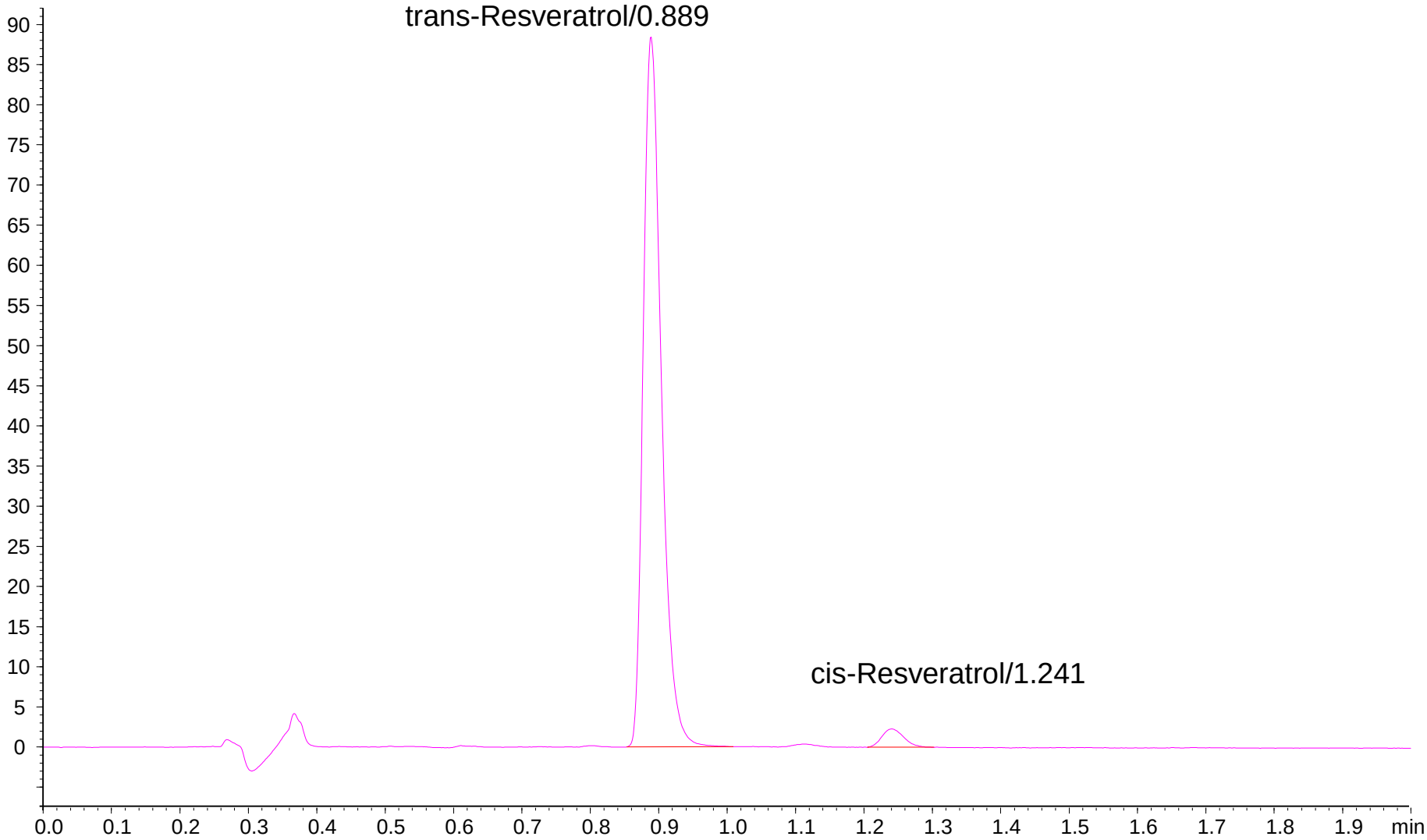
Detection: RF-20AXS – Excitation λ = 300 nm Emission λ = 386 nm SENS = Med

Cell Temperature: 20 °C

Inj. Vol.: 2 µl

Results

- 17.5 mg /L trans-resveratrol standard – PDA detection 291 nm



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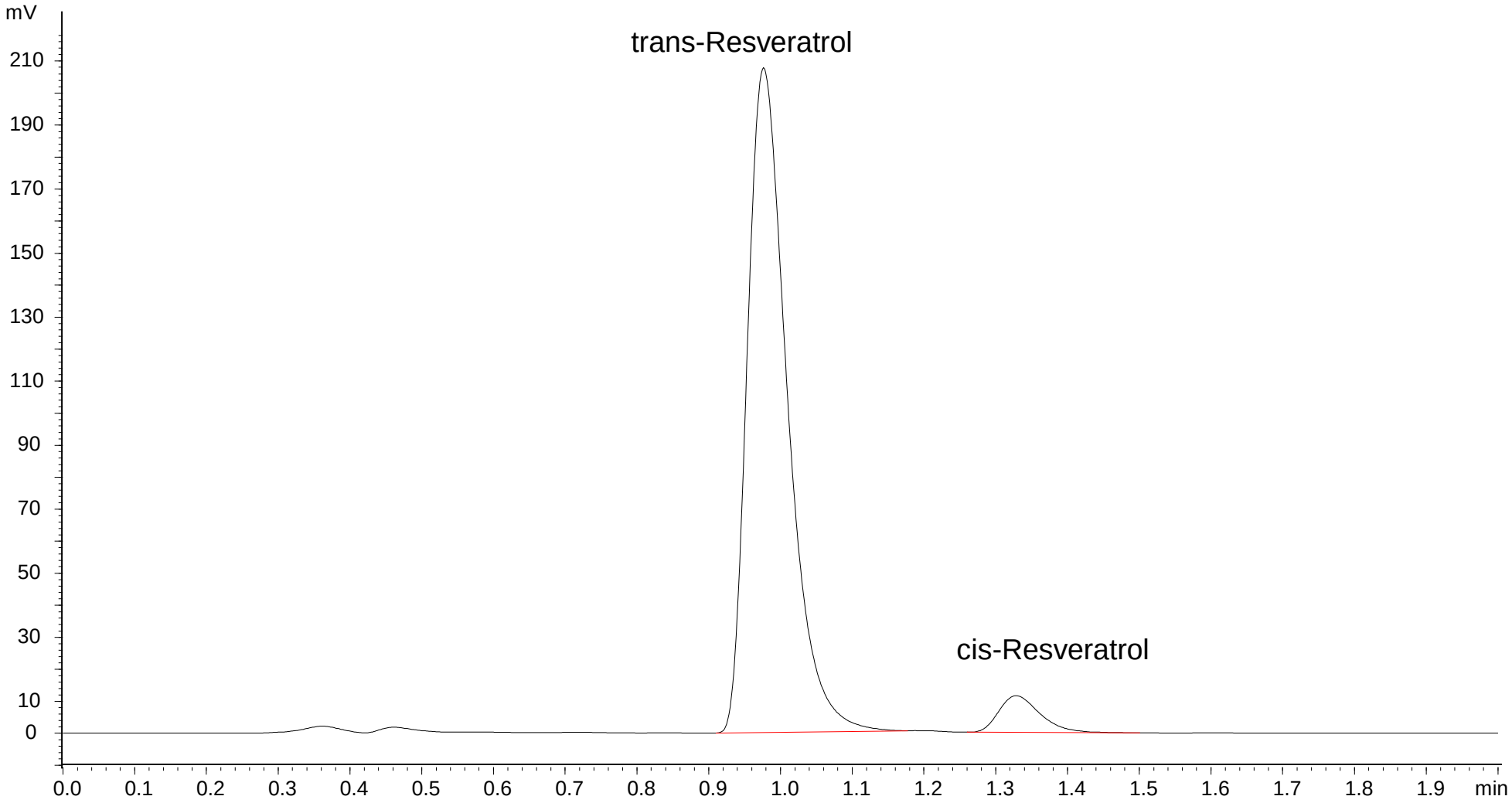
Results

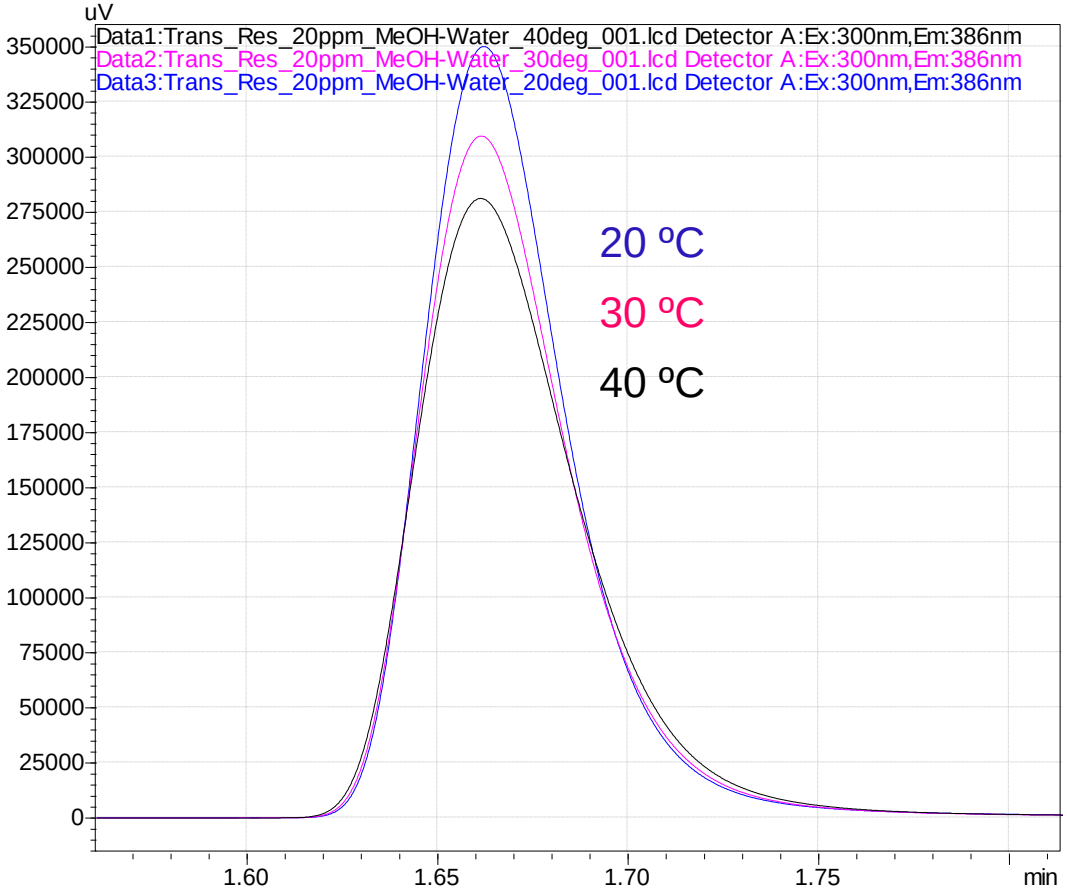
- 9 mg /L trans-resveratrol standard – RF - Ex:300nm Em:386nm



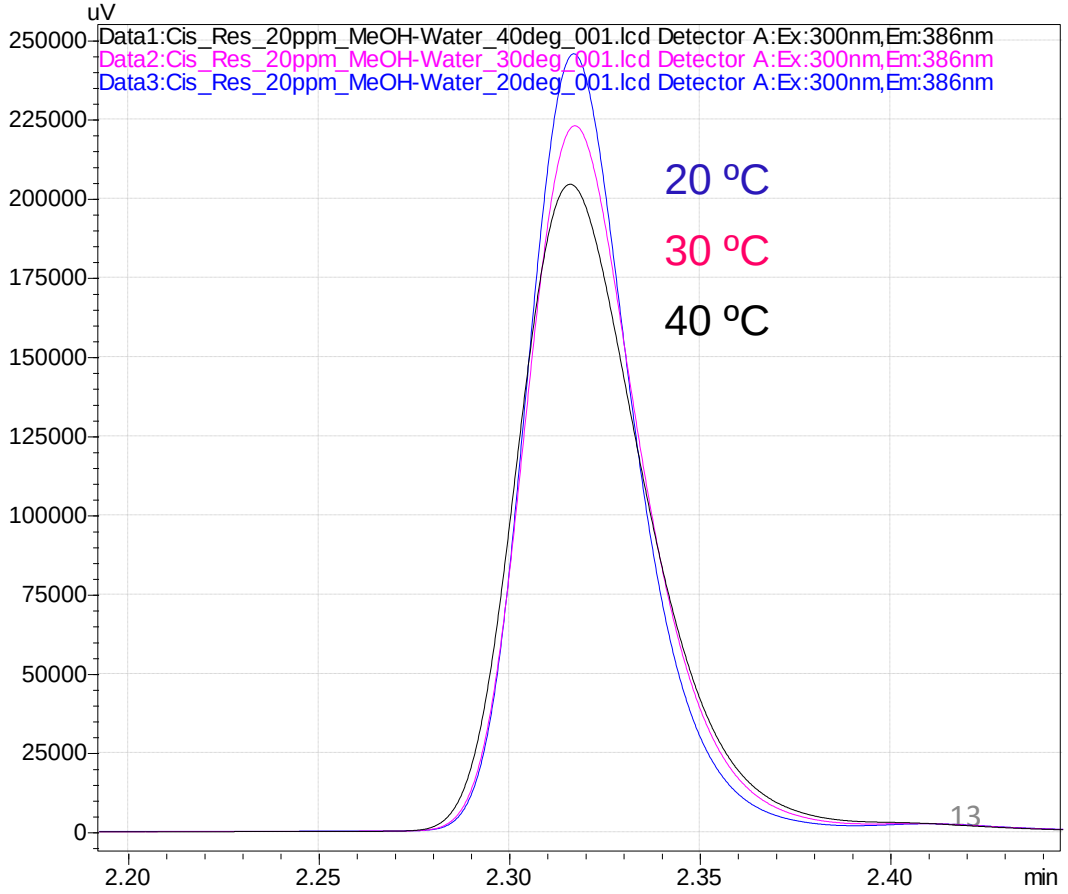
Method Development

- Cell temperature affects sensitivity
  - improve baseline stability and eliminate the effect of temperature

trans-Resveratrol



cis-Resveratrol



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Sample preparation

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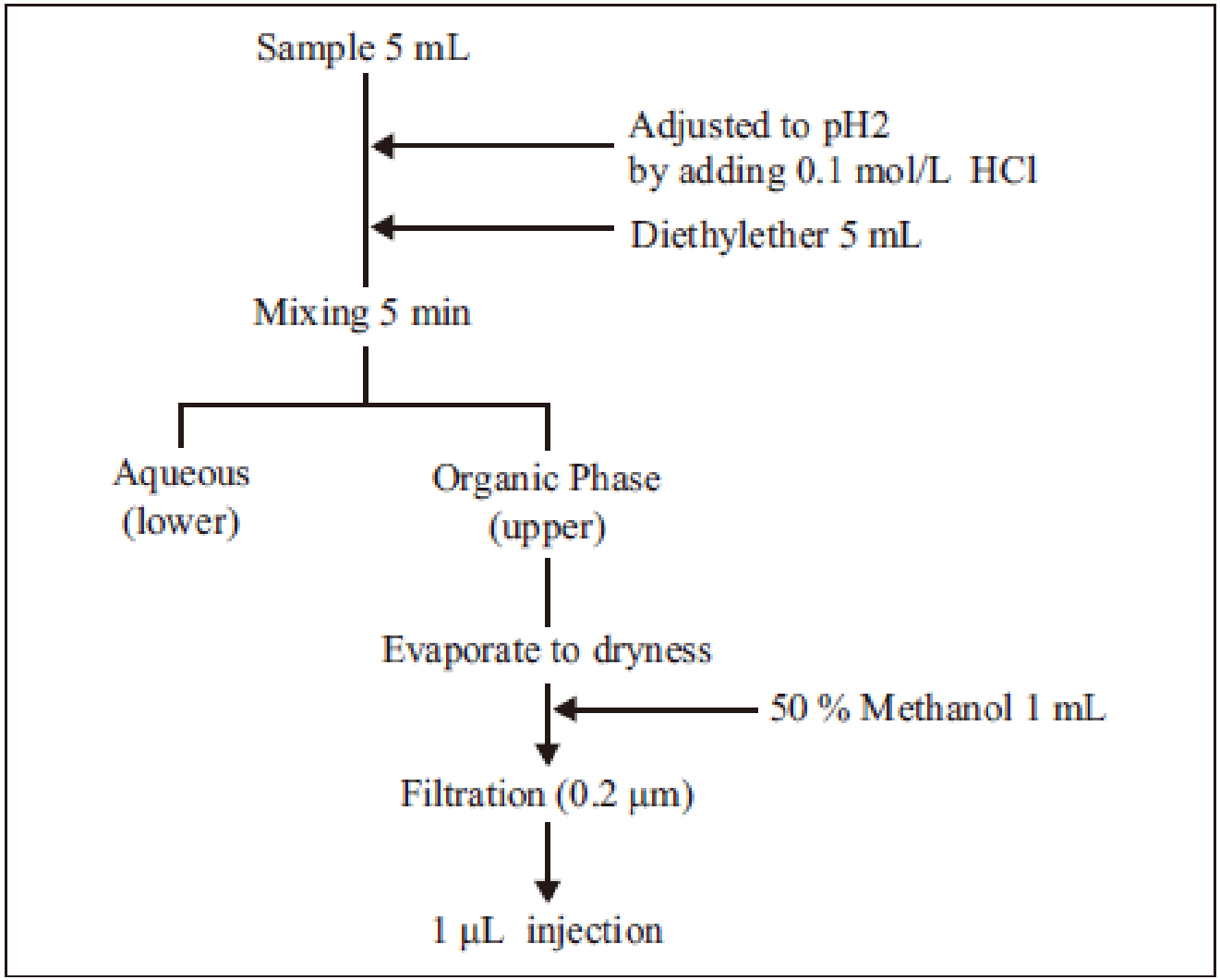
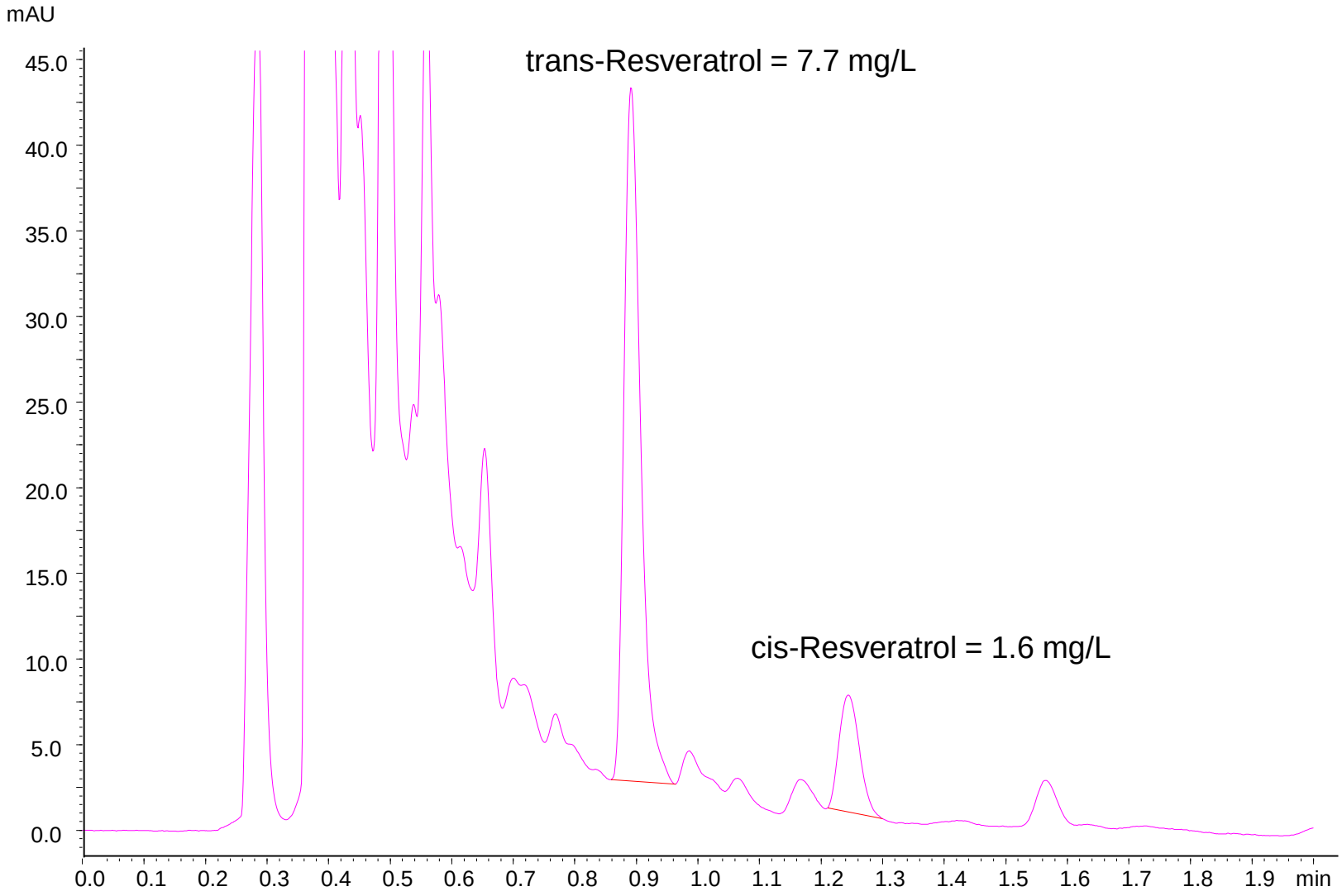
Sample preparation

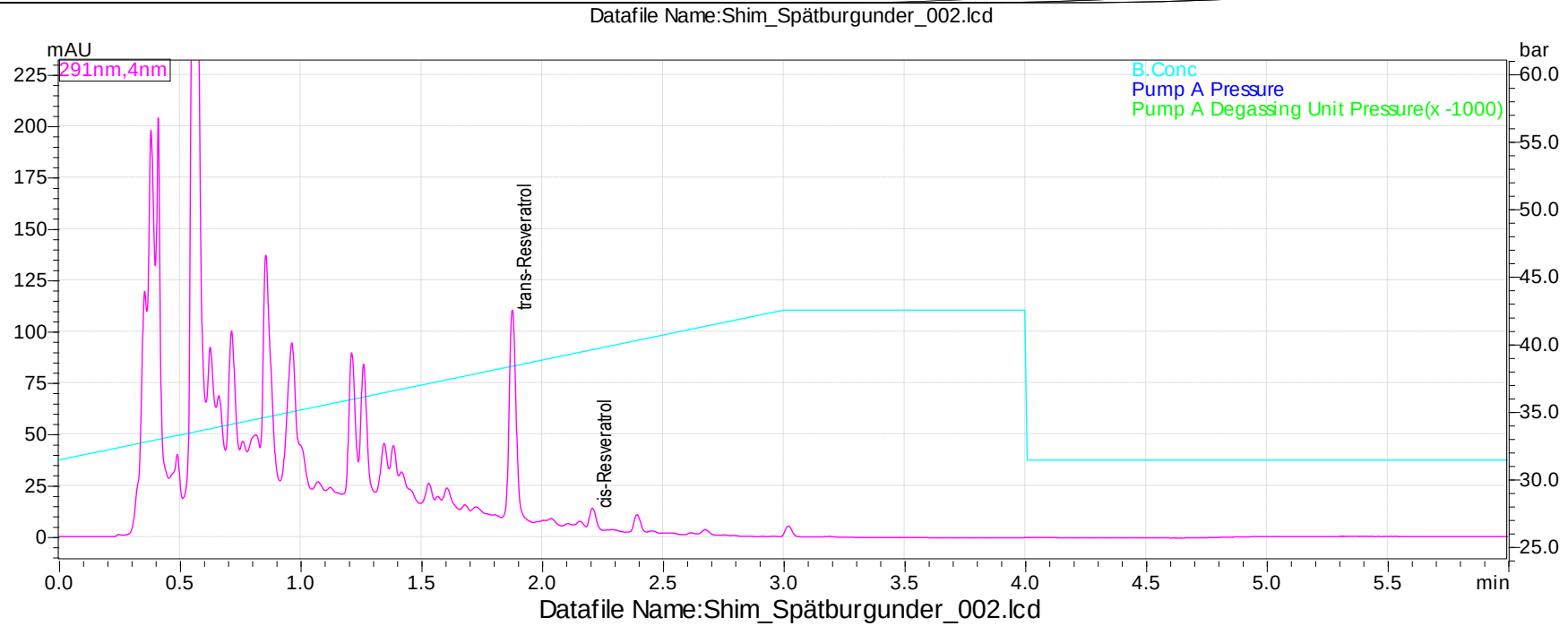


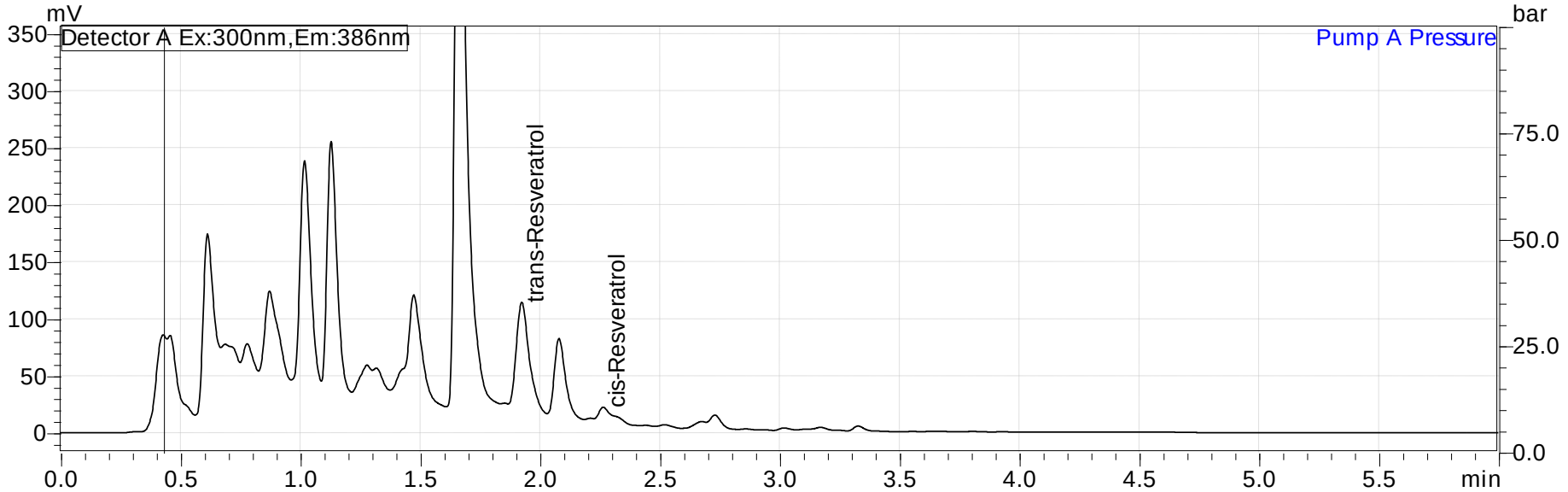
Results

- Wine sample, LL extracted – PDA detection 280 nm



Example: Filtered red wine





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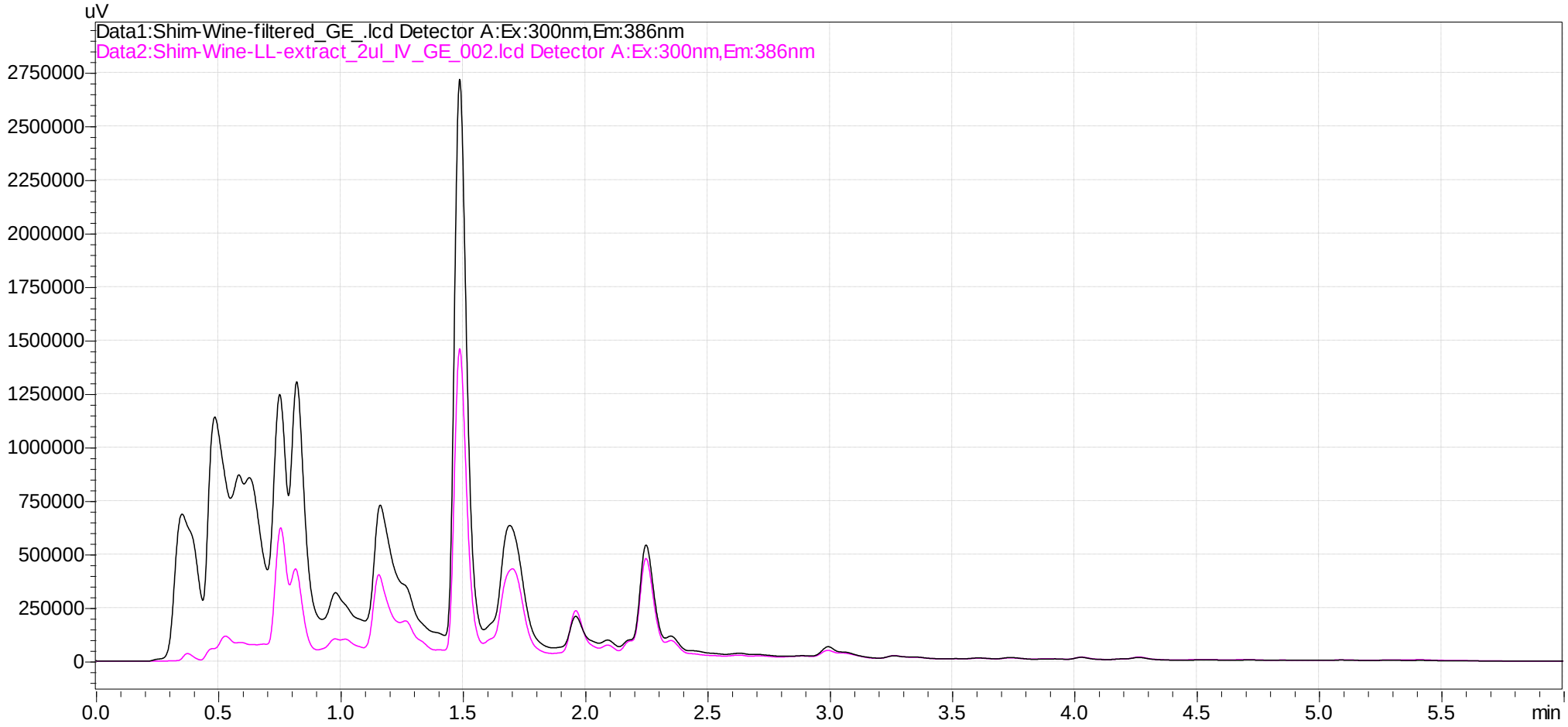
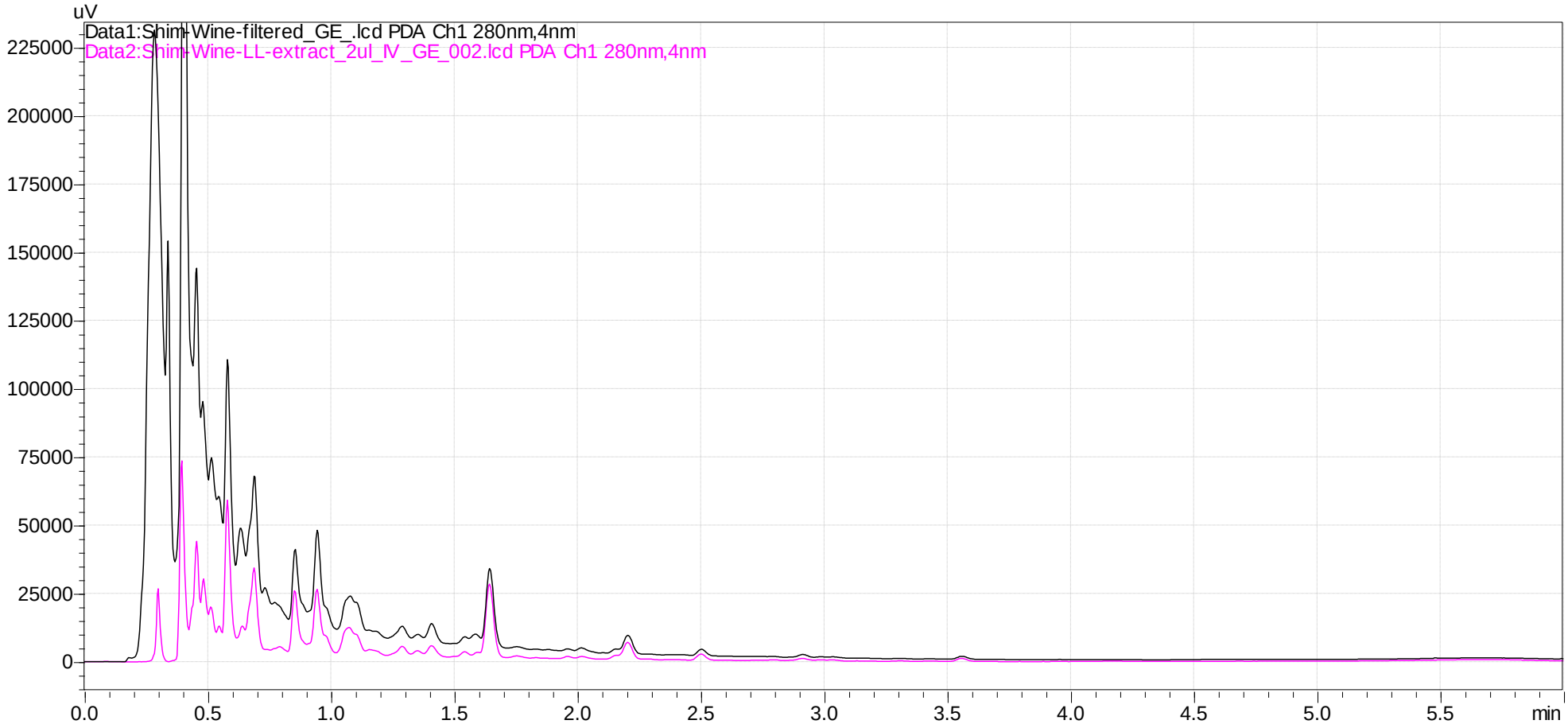
LL extraction vs filtered (0,2 µm) sample RF Signal



LL extraction vs filtered (0,2 µm) sample PDA signal



Conclusion

- The proposed method allows the determination of trans-resveratrol in wine in a one step procedure
- Fluorescence detection is highly sensitive and allows for the detection of resveratrol in concentrations present in red wine
- Animal studies suggest as much as 500 mg daily may be necessary to provide any health benefits, even if a 40 mg daily dose is sufficient, as suggested elsewhere, red wine contains at most 12 mg resveratrol per liter, you'd need to drink a little over 3 liters of wine daily to get that much resveratrol.

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Cheers !