A fast, scalable and selective method for total tannin determination in wine

Ágnes Dienes-Nagy[1*], Daniel Bátora [2], Liming Zeng[3], Christian E. Gerber[2], Jérôme P. Fischer[2], Martin Lochner[2], Jürg Gertsch[2]

1. Agroscope, 1260 Nyon, Switzerland
2. Institute of Biochemistry and Molecular Medicine, University of Bern, 3012 Bern, Switzerland
3. Changins, Viticulture and oenology, HES-SO University of Applied Sciences and Arts Western Switzerland

**presenting author*

Introduction and goals

Conventional tannin quantification methods are primarily colorimetric, some of which suffer from a high interference with anthocyanidins and therefore are unsuitable for many emerging disease resistant grape varieties. Even though precipitation-based methods remain a relatively good proxy for astringency they are difficult to scale to large number of samples.

Fluorescence or chemiluminescence-based assays could provide a more practical alternative by offering high sensitivity while minimizing interference artefacts. A new technique based on rhodamine-quenching was applied to quantify condensed tannins present in wine and was compared to a methylcellulose precipitation and acidic butanolysis[1].

Chemical structure and fluorescence quenching of fluorophores

Fig. 1: Structure activity relationship (SAR) A) rhodamine-based fluorophores and B) silicon-rhodamine (SiR) conjugates on tannic-acid (TA) induced fluorescence quenching.

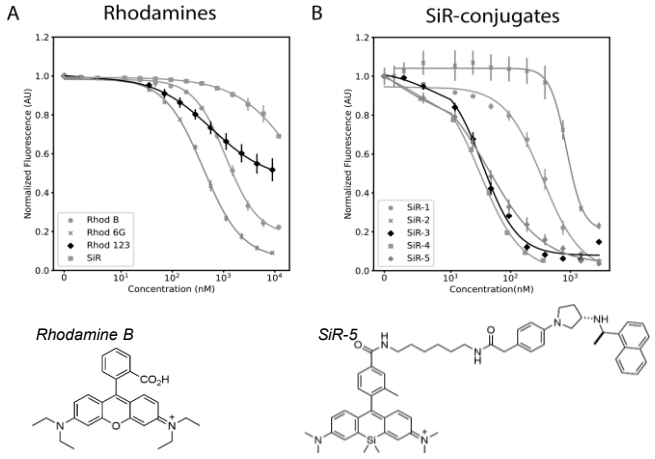


Table 1: Key parameters of two rhodamine fluorophores including the excitation maxima (λ_{ex}), limit of detection for tannic acid (LOD), the IC50 value of quenching and the linear range for tannic acid (LR).

	Rhodamine B	SiR-5
λ_{ex} (nm)	545	650
LOD (nM)	90	0.5
IC_{50} (nM)	1117±51	42.5±3
LR (nM)	94-3000	5-100

Selectivity

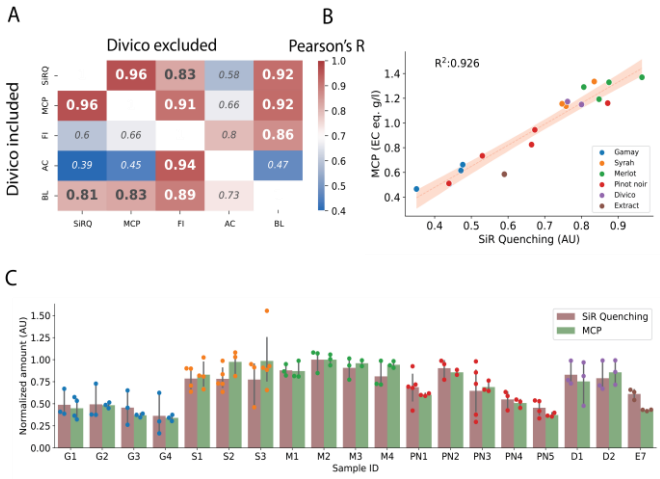
Table 2: Selectivity of SiR-5 towards structurally diverse tannins and anthocyanins measured by the IC50 value of fluorescence quenching.

Compound	IC_{50} (nM ± STD)	Compound	IC_{50} (nM ± STD)
TA	34±2	PB2	7942±859
PGG	105±3	M36G	19427±4188
C3G	462±56	M3G	33369±4961
E3G	1197±386	Punicalagin	N/A
C5G	2389±399	Vescalagin	N/A
GC3G	2563±773	GA	N/A
EGC3G	2571±345	EC	N/A
PA1	2618±494	GC	N/A
TGG	3882±1836	C	N/A
PA2	7370±3072		

Tannin determination in wine

Tannin quantification was carried out in 17 wine samples with SiR-quenching and was compared with conventional methods that included methylcellulose precipitation (MCP), butanolysis (BL), the Folin-Ciocalteu index (FI) and the anthocyanin content (AC).

Fig. 2: A) Heatmap depicting the Pearson's correlation coefficients for all methods, B) Linear regression between MCP and SiR-quenching, C) Comparison of variability between MCP and SiR-quanching in wine.



Conclusion

A fast assay with high correlation to methylcellulose precipitation (R^2 =0.92) was developed and validated that allows the sensitive and selective quantification of tannins.

The assay was two orders of magnitude more sensitive towards certain wine catechins than to malvidins, making it suitable for wines with high anthocyanin content like new resistant grape varieties.

The assay can be adapted to microplates for high throughput analysis.

[1] Bátora D, Dienes-Nagy Á, Zeng L, et al. Hypersensitive quantification of major astringency markers in food and wine by substoichiometric quenching of silicon-rhodamine conjugates. *Food Chem X*. 2024;23:101592. Published 2024 Jun 25. doi:10.1016/j.fochx.2024.101592