SFC and CE – a comparison of two orthogonal methods for the analysis of dihydrochalcones in apple leaves

SUPPLEMENTARY INFORMATION

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1. SFC method development

1.1. Stationary phase

Table S1. Stationary phases evaluated in this study.

Column	Dimensions	Particle size
Viridis® BEH 2-EP	3 mm x 100 mm	1.7 µm
Viridis® BEH	3 mm x 100 mm	1.7 µm
Viridis® HSS C18 SB	3 mm x 100 mm	1.8 µm
Torus™ 1-AA	3 mm x 100 mm	1.7 µm
Torus™ DIOL	3 mm x 100 mm	1.7 µm
Torus™ DEA	3 mm x 100 mm	1.7 µm

1.2. Influence of individual SFC parameters on the separation

Stationary phase

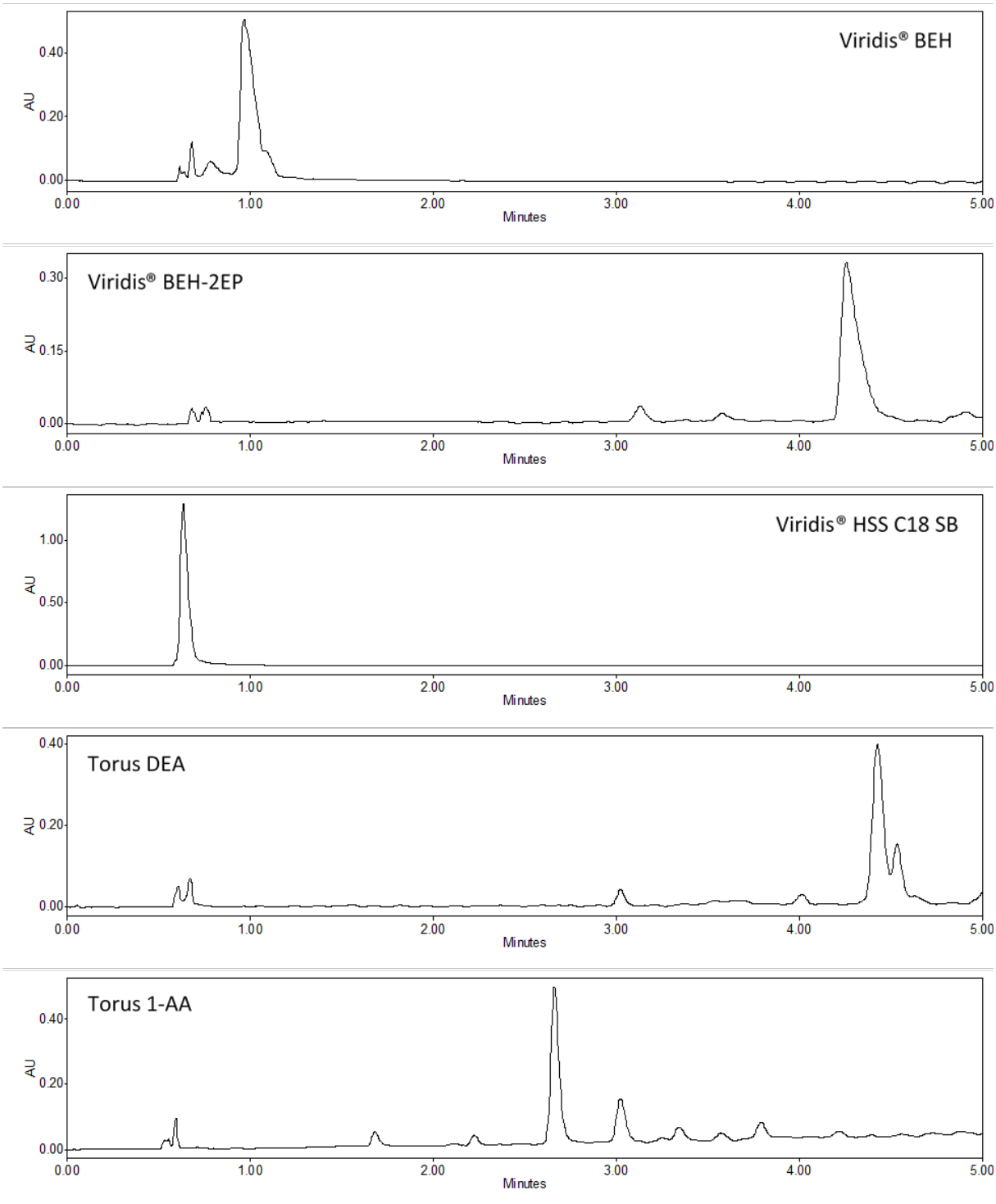

Figure S1. Impact of stationary phase on the separation of the test solution; other settings were optimal.

Modifier and Additives

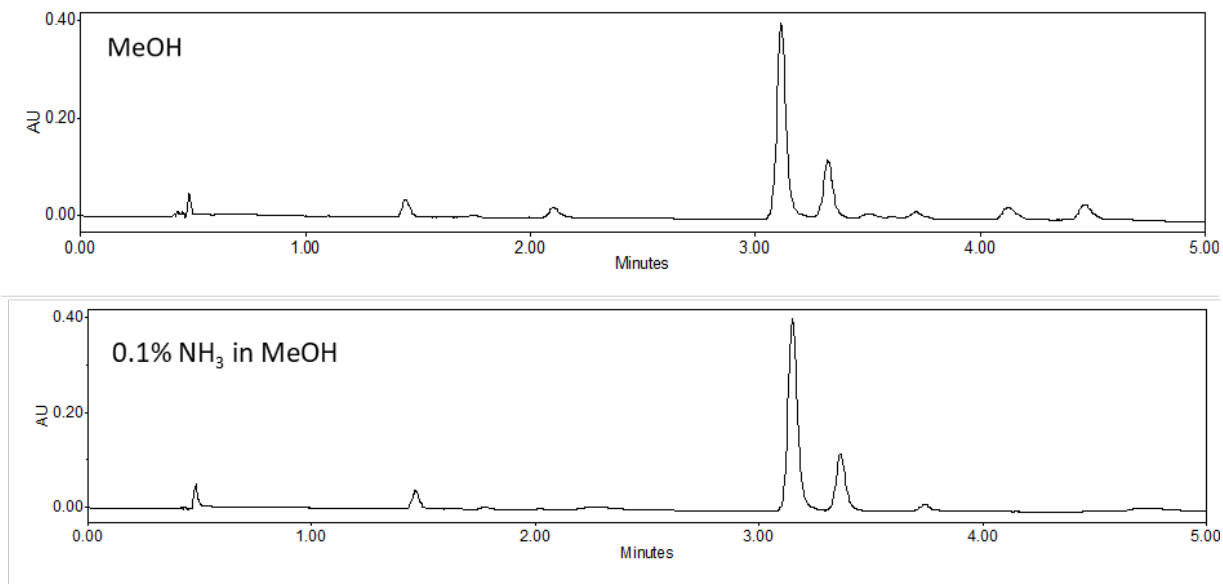

Figure S2. Impact of methanol and methanol with ammonia as modifier on the separation of the test solution; other settings were optimal.

Temperature

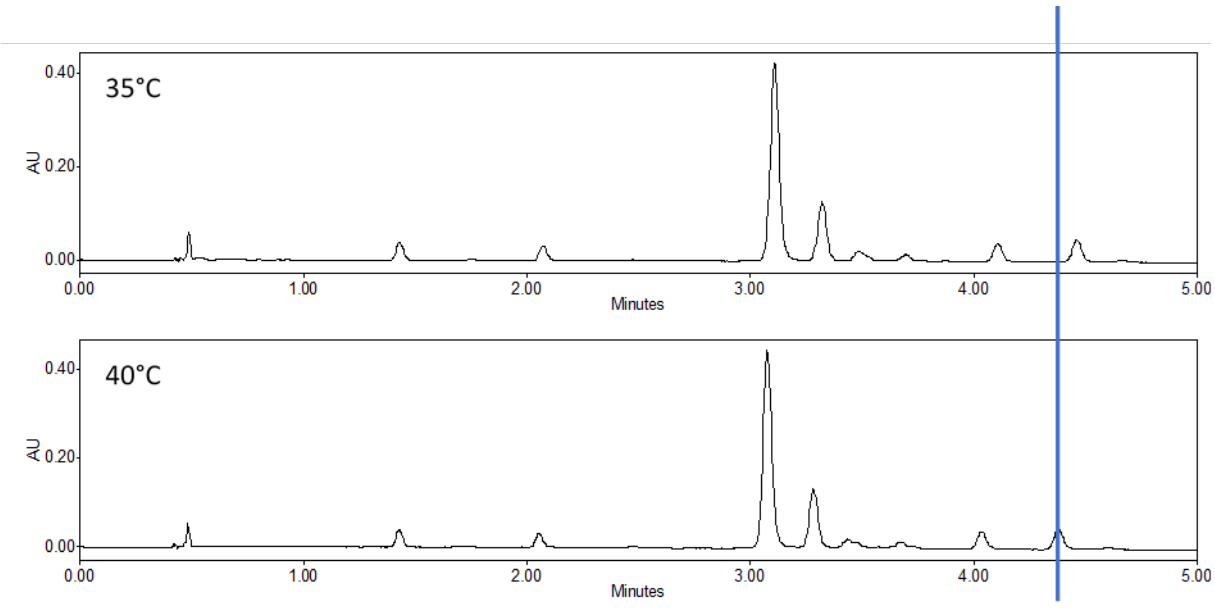

Figure S3. Impact of temperature on the separation of the test solution; other settings were optimal.

Flow rate

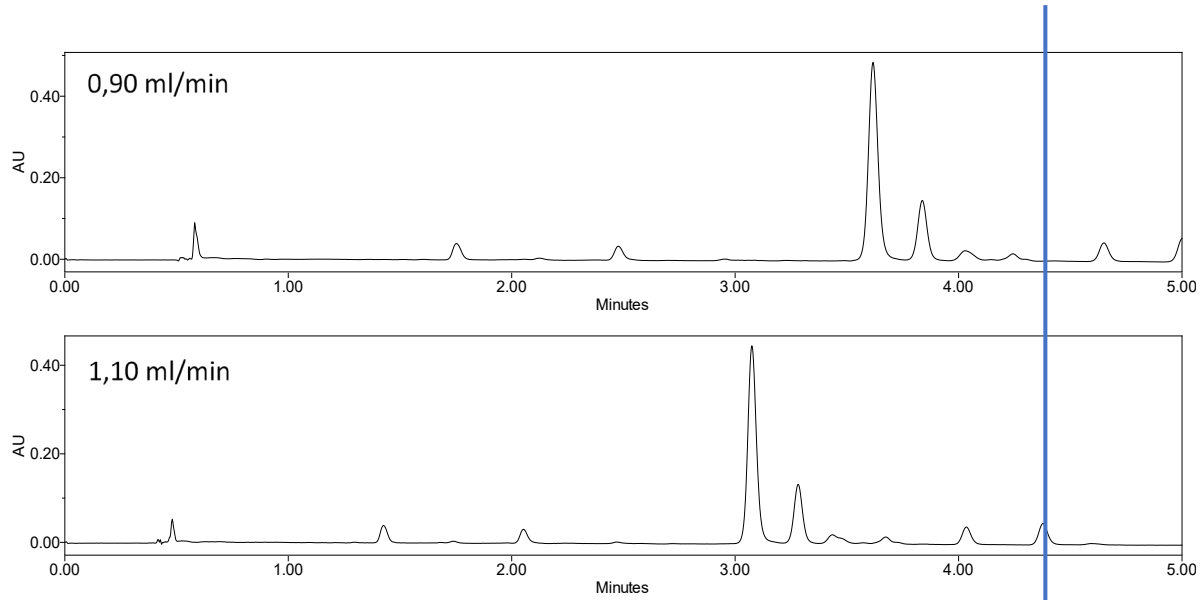

Figure S4. Impact of different flow rates on the separation of the test solution; other settings were optimal.

ABPR

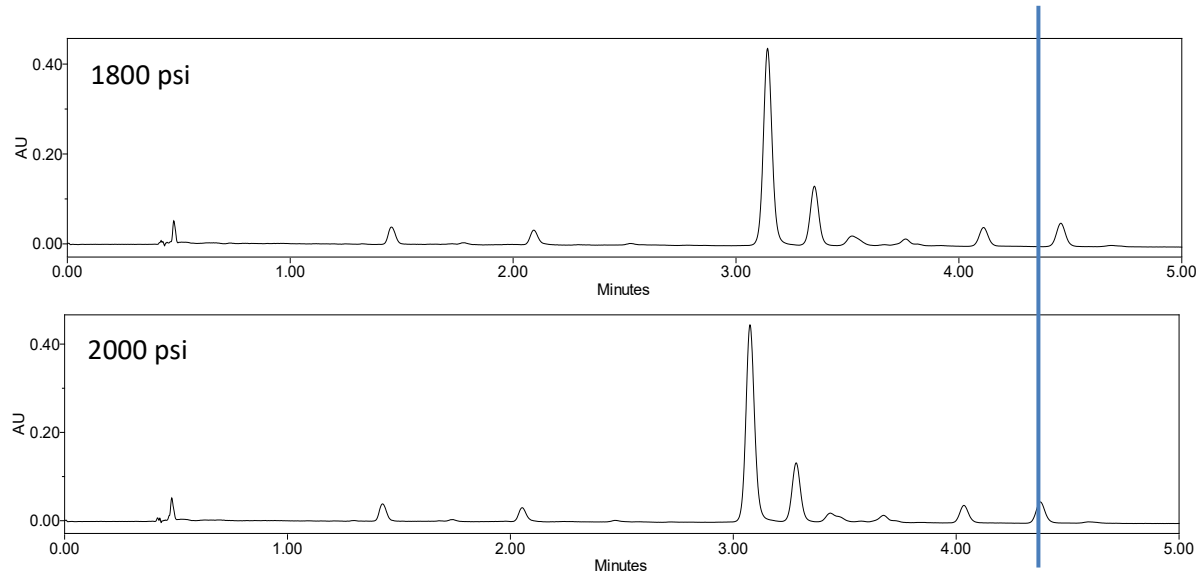

Figure S5. Impact of backpressure on the separation of the test solution; other settings were optimal.

Gradient

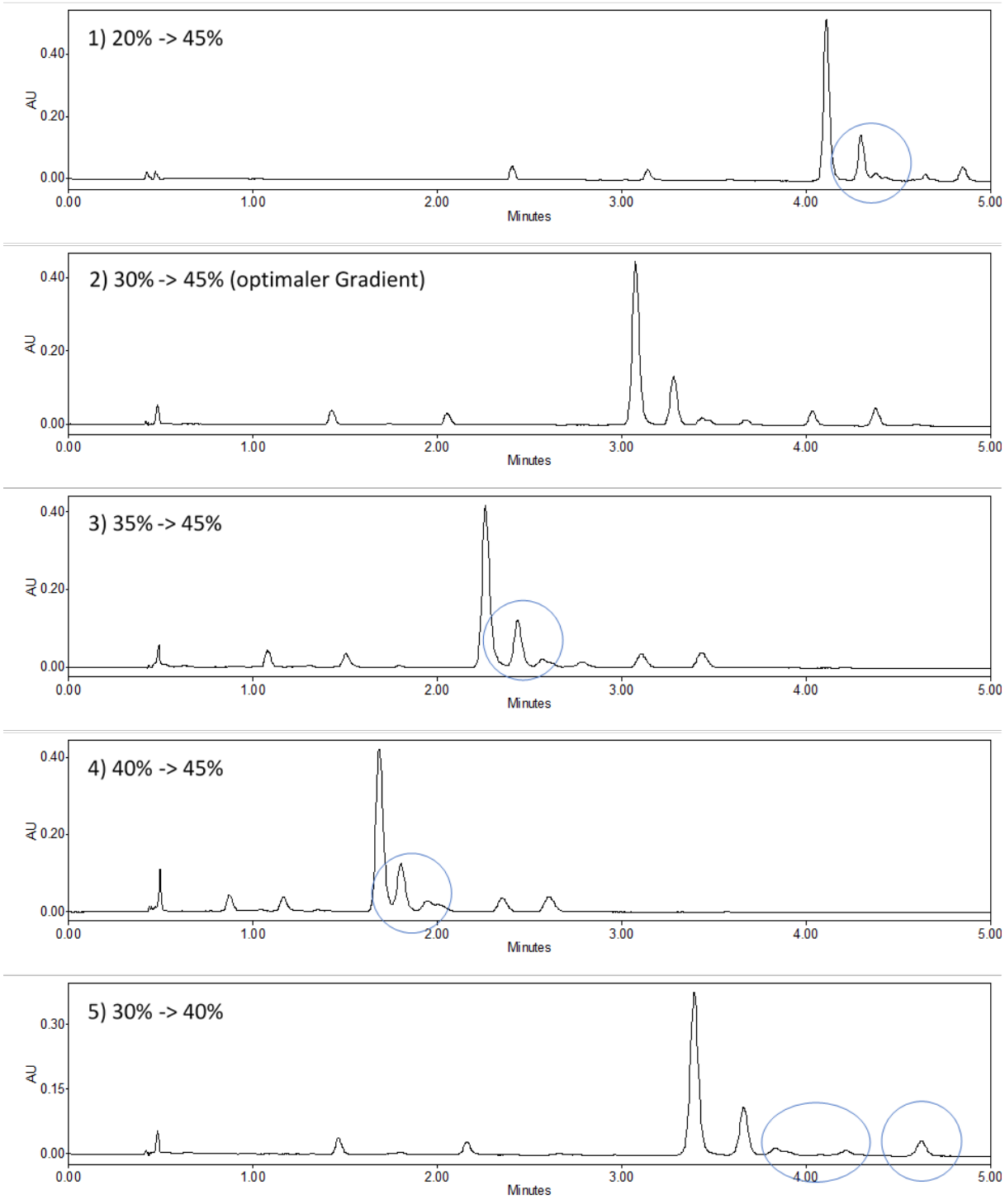

Figure S6. Impact of the gradient on the separation of the test solution; other settings were optimal

2. CE

Phosphate Buffer

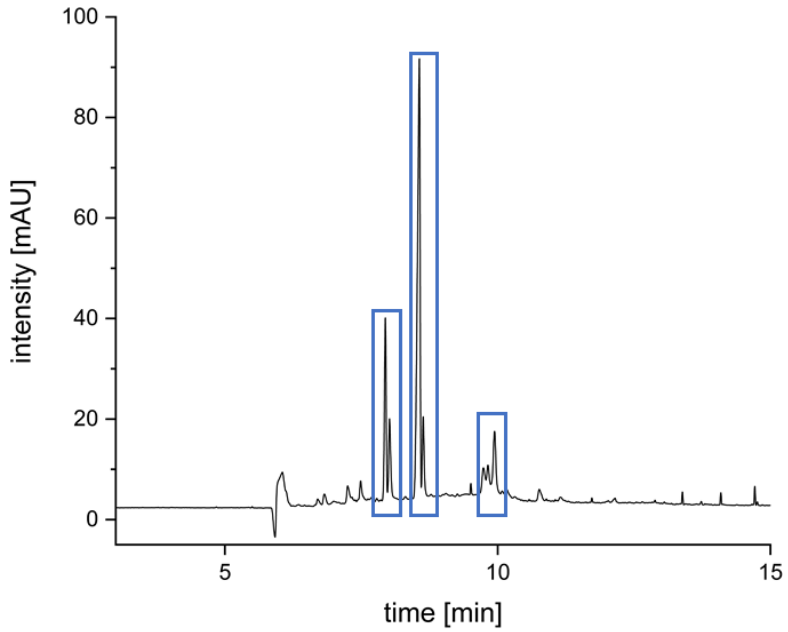

Figure S7. Separation of the test solution with a phosphate buffer of pH 8.50. Severe coelution (marked in blue) can be observed concerning all target DHCs.

pH-Value

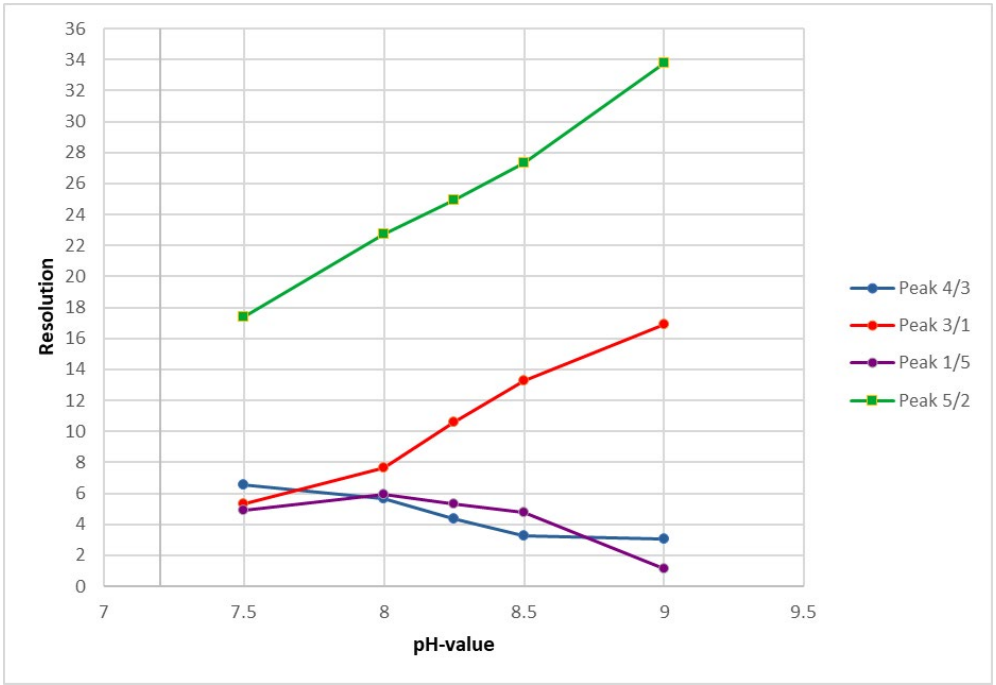

Figure S8. Resolution of the target analytes at different pH values (all borate buffers).

Voltage

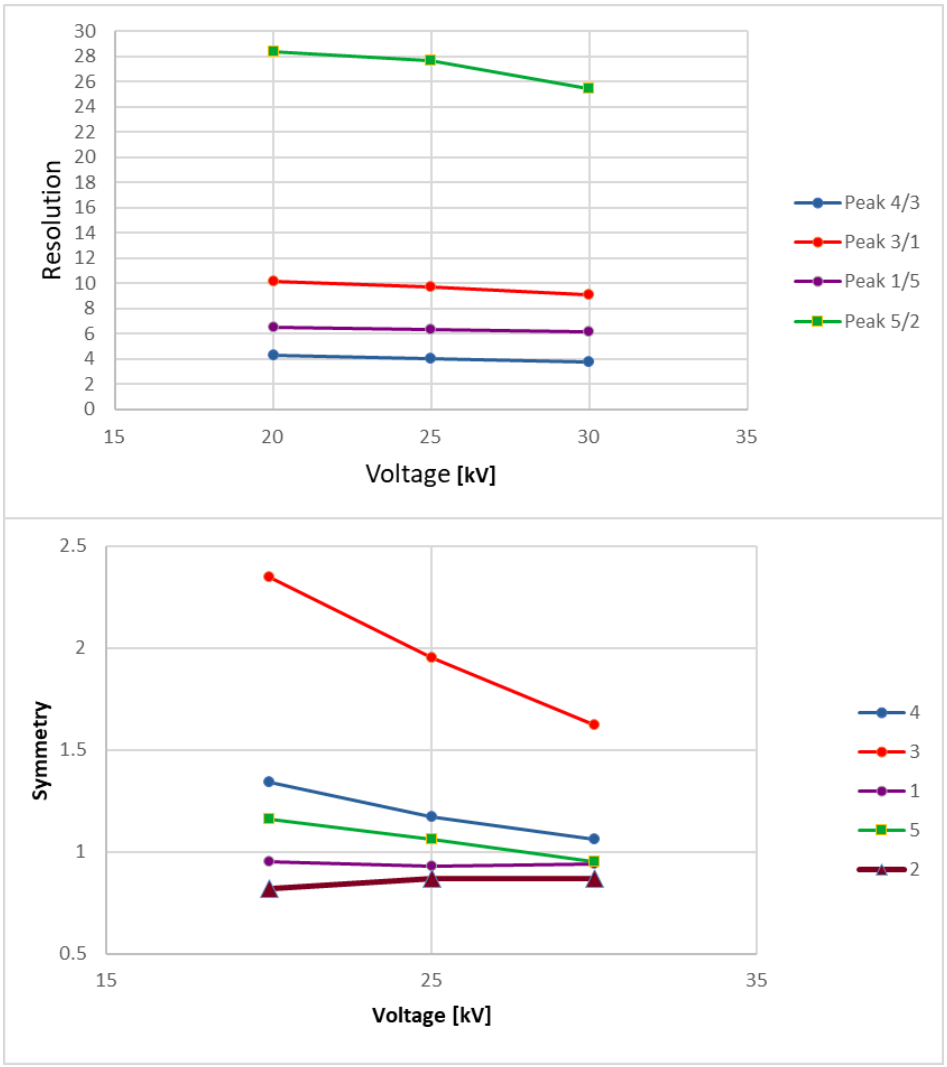

Figure S9. Resolution and peak symmetry of the target analytes at different voltages.

Temperature

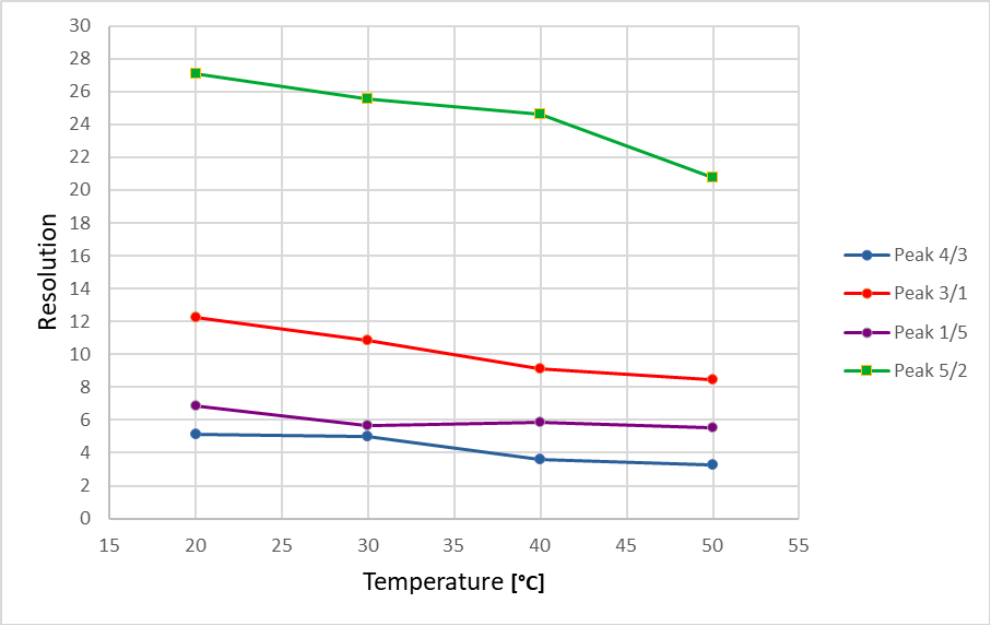

Figure S10. Resolution of the target analytes at different temperatures.

Organic additives

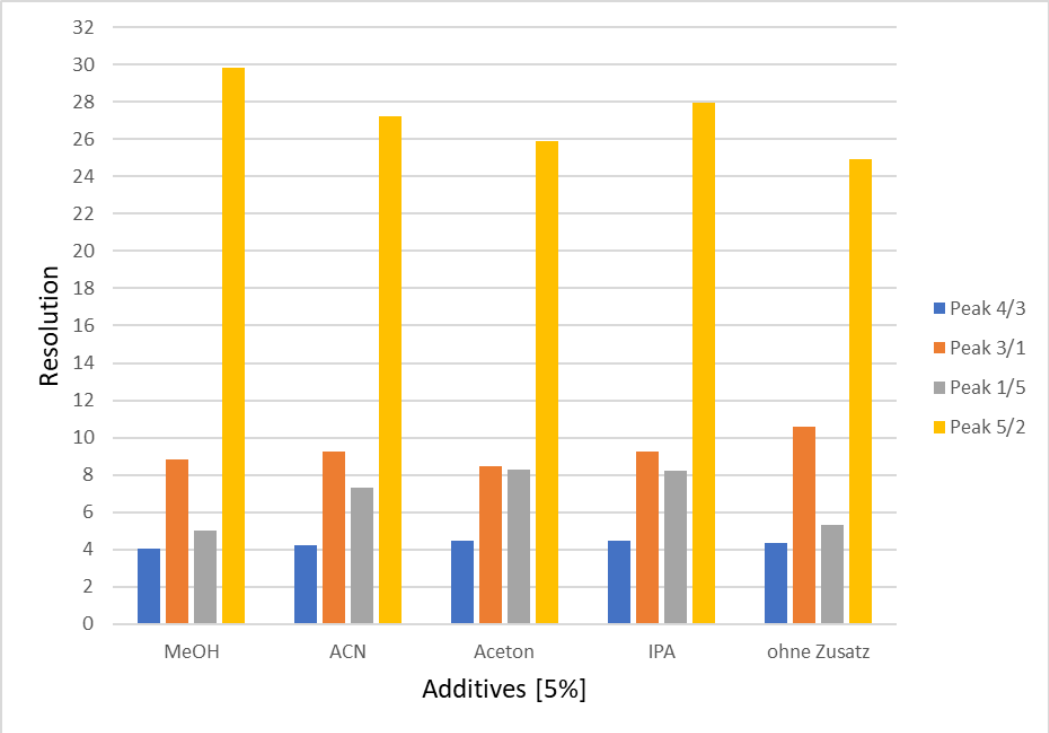

Figure S11. Resolution of the target analytes using different organic additives.

3. UV-spectra of the DHCs

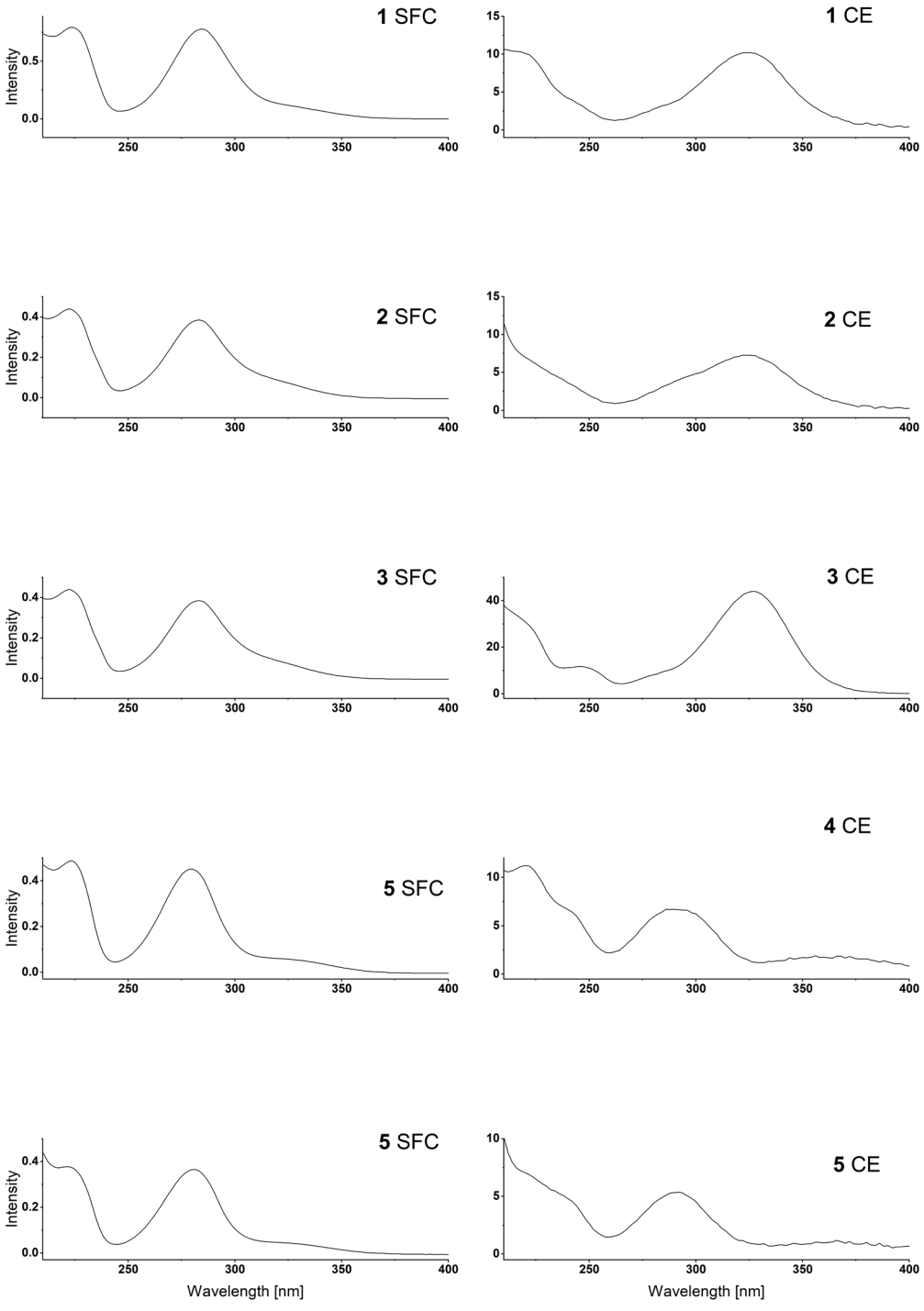

Figure S12. UV-spectra of the 5 DHCs in comparison, as determined in sample solutions (concentrations of compounds not identical); left: SFC, right: CE.

4. Analysis of the samples using SFC

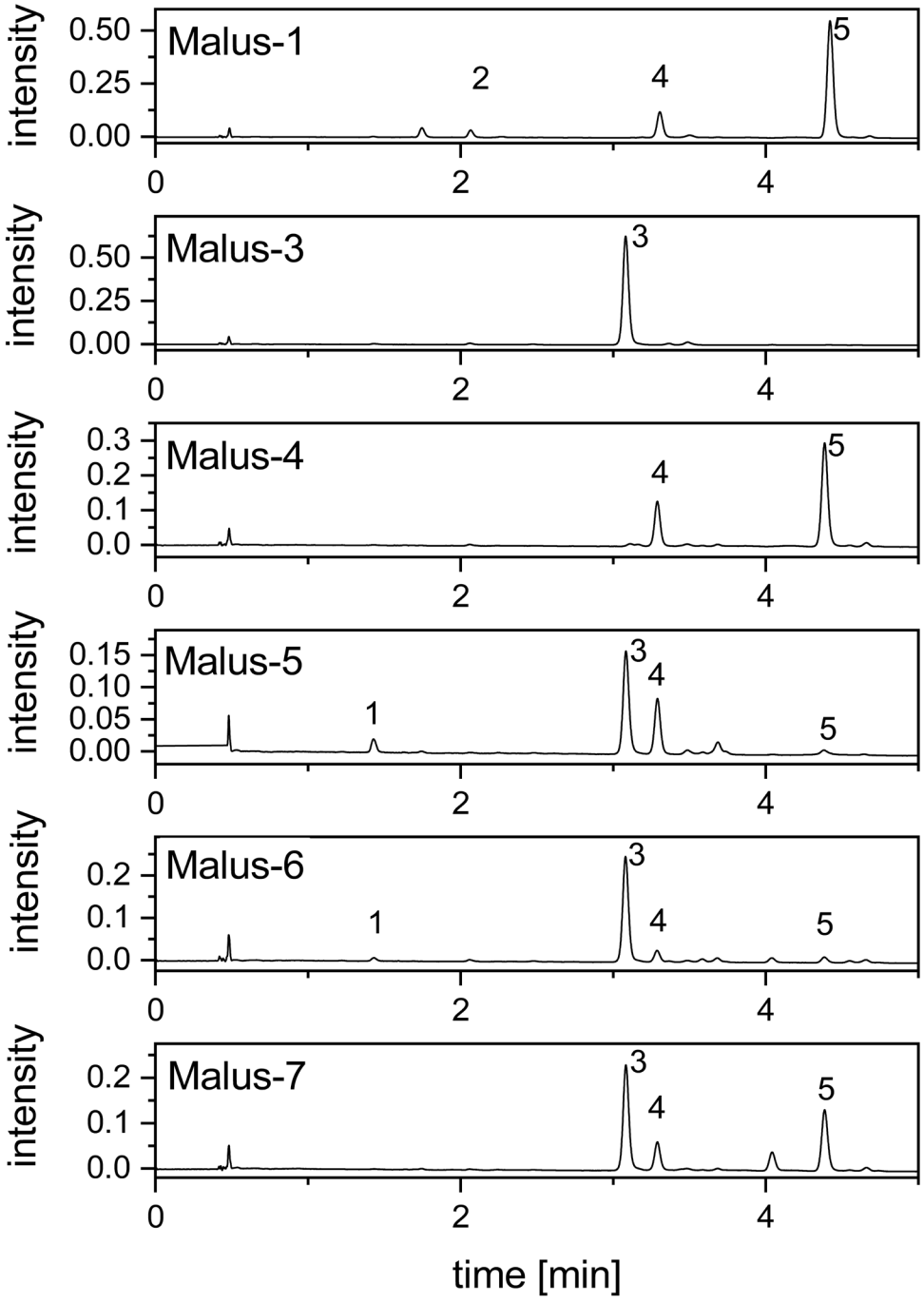

Figure S13. SFC chromatograms of the analyzed samples not shown in the manuscript under optimized conditions.

5. Analysis of the samples using CE

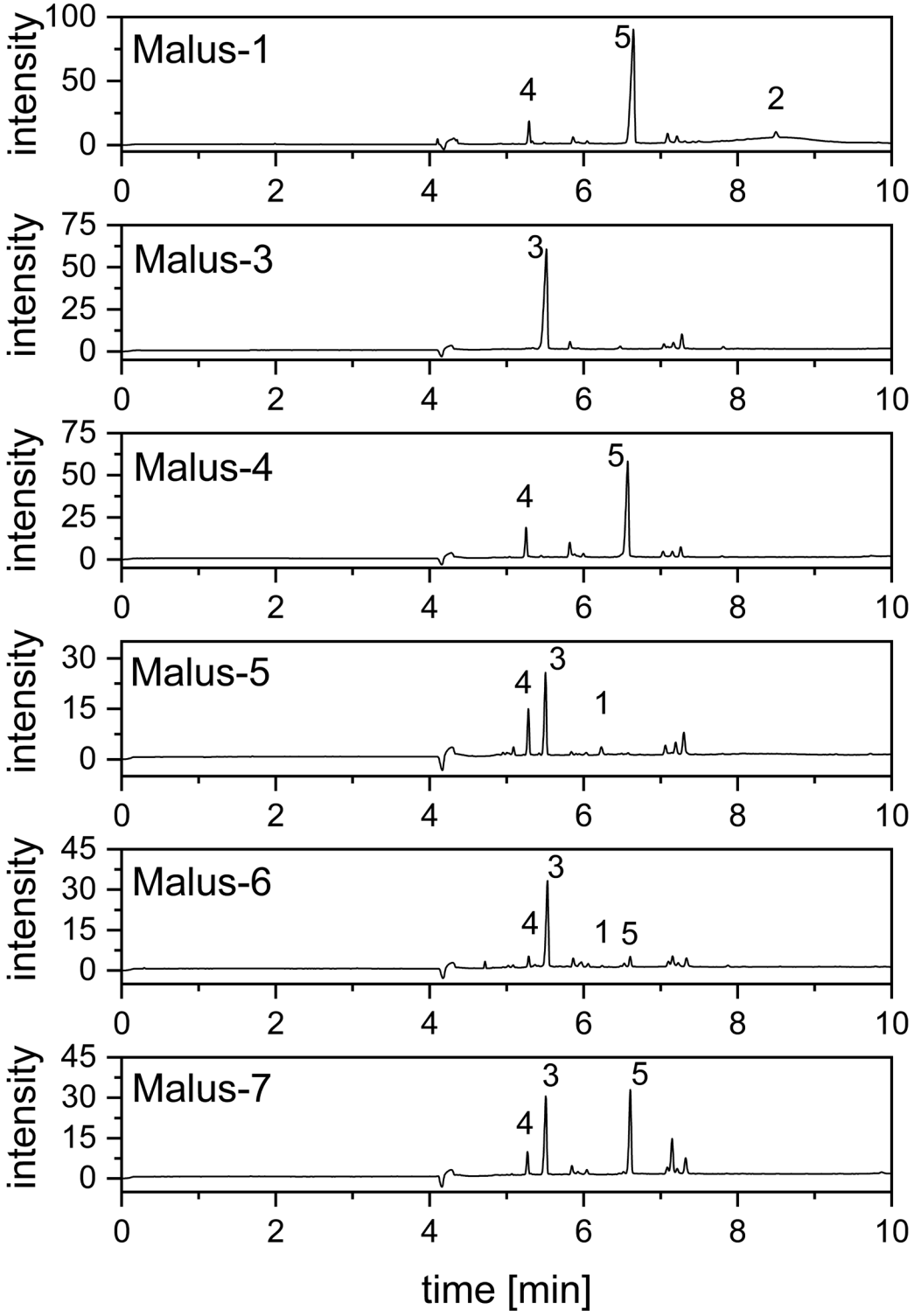

Figure S14. CE electropherograms of the analyzed samples not shown in the manuscript under optimized conditions.

6. Statistical evaluation

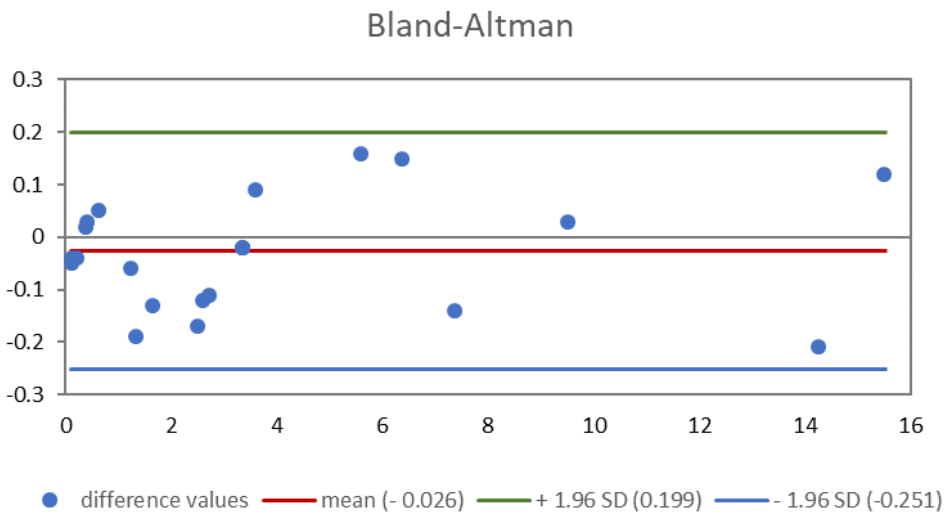

Figure S15. Statistical method comparison using a Bland-Altmann plot.

7. Selection of samples

Table S2. Detailed information on the samples analyzed in this study.

Sample	Species	Tree ID	Provenance	Time of harvest
Malus-1	*Malus micromalus*	31	Fondazione Edmund Mach - San Michele all' Adige	August 28, 2017
Malus-2	*Malus prunifolia*	60	Fondazione Edmund Mach - San Michele all' Adige	August 28, 2017
Malus-3	*Malus huphensis*	131	Fondazione Edmund Mach - San Michele all' Adige	August 28, 2017
Malus-4	*Malus toringo*	136	Fondazione Edmund Mach - San Michele all' Adige	August 28, 2017
Malus-5	*Malus baccata*	189	Fondazione Edmund Mach - San Michele all' Adige	August 28, 2017
Malus-6	*Malus sikkimensis*	246	Fondazione Edmund Mach - San Michele all' Adige	August, 2022
Malus-7	*Malus floribunda*	279	Fondazione Edmund Mach - San Michele all' Adige	August 28, 2017