Supporting Information to:

Evaluation of Polyphenol Composition in Red Leaves from Different Varieties of Vitis vinifera Species

Ernst Schneider¹
Holger von der Heydt²
Anke Esperester²

Affiliation
1 Dr. Ernst Schneider PhytoConsulting, Marklkofen-Freinberg, Germany
2 Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany

Correspondence
Dr. Ernst Schneider
PhytoConsulting
Seeblick 11
Freinberg
84163 Marklkofen
Germany
Phone: +49-8734-938214
Fax: +49-8734-938215
E-mail: schneider.e@phyto-consulting.de
Validation of Analytical Methods

Methodology applied is according to ICH Guideline Q2(R1).

For assay of content it is necessary to characterise

- Specificity
- Linearity
- Range
- Accuracy
- Precision, Repeatability

Assay of flavonol content

Sample preparation
2.0 g of powdered red vine leaves were heated with 40 mL methanol 70% for 15 minutes under reflux, filtered after cooling and made up to 100 mL with methanol 70%. As reference solution 15 mg of isoquercitrin were dissolved in 20.0 mL methanol. 2.00 mL of the solution were diluted to 10.00 mL with mobile phase.

Apparatus and analytical conditions
Standard HPLC equipment consisting of an HPLC pump with column oven and UV detector. The assay was carried out with an injection volume of 10 µL using a Superspher RP 8 4µm column with column temperature of 25°C. The mobile phase was an isocratic eluent mixture of 10.7 g tetrahydrofuran, 5.5 g acetonitrile and 81 g phosphoric acid 0.003 mol/L. The flow rate was 1 mL/min and detection wavelength 360 nm. The total flavonoid content was calculated as quercetin 3-O-β-D-glucuronide using the conversion factor 1.1. A typical HPLC chromatogram of flavonoids in red vine leaves is shown in Fig. 2 in the manuscript.

Specificity
Specificity of the three main peaks of the HPLC chromatogram was tested by their retention time and identified by reference substances. A representative chromatogram is presented in Fig. 3.

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention Time [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoquercitrin</td>
<td>12.0 – 12.8</td>
</tr>
<tr>
<td>Quercetin 3-O-β-D-glucuronide</td>
<td>14.1 – 15.1</td>
</tr>
<tr>
<td>Kämpferol 3-O-glucoside</td>
<td>16.1 – 17.2</td>
</tr>
</tbody>
</table>

Linearity
Linearity in respect of total flavonoid content, determined on a batch of the extract preparation.

The linearity and range of the method are established at concentrations between 25 and 150% of the theoretical concentration of sample in the test solution.
### Calibration Plot

#### Linearity

Range:

Linearity was tested over a range of concentrations between 25 and 150% of the theoretical concentration of sample in the test solution.

So derived from linearity study the necessary minimum range of 80 to 120 percent of test concentration can be specified.

Accuracy:

Accuracy can be inferred because precision, linearity and specificity have been established.
**Precision, repeatability**

*Precision of the content of total flavonols calculated as Quercetin 3-O-β-D-glucuronide*

<table>
<thead>
<tr>
<th>Determination No.</th>
<th>Injection 1 [%]</th>
<th>Injection 2 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.741</td>
<td>1.747</td>
</tr>
<tr>
<td>2</td>
<td>1.836</td>
<td>1.837</td>
</tr>
<tr>
<td>3</td>
<td>1.845</td>
<td>1.846</td>
</tr>
<tr>
<td>4</td>
<td>1.777</td>
<td>1.780</td>
</tr>
<tr>
<td>5</td>
<td>1.843</td>
<td>1.848</td>
</tr>
<tr>
<td>6</td>
<td>1.757</td>
<td>1.762</td>
</tr>
</tbody>
</table>

Mean of both injection: 1.802%

Confidence interval (P = 0.95) = [1.773 – 1.829]

Standard deviation [%] = 0.040

Relative standard deviation (coefficient of variation) [rel. %] = 2.45

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**Robustness**

Typical general variations are:

- stability of analytical solutions
  The reference solution of isoquercitrin was stored at room temperature for 11 days in transparent and brown glass vials. In addition sodium hydroxide, hydrochloric acid and hydrogen peroxide was added. The solution was also stored for 2 hours in xenon lamp light and 48 hours at 40°C. In either case no degradation was observed.

- recovery test
  Recovery was tested at concentrations of 25, 50, 75, 100, 125 and 150% of the theoretical concentration of sample in the test solution. The samples tested and the results found are summarised in the tables below.

Each sample was subjected to three separate work-ups and the resulting solutions were each injected once. The results were subjected to statistical analysis.

**Recovery of total flavonol content**

<table>
<thead>
<tr>
<th>Sample concentration as % of theory</th>
<th>Recovery, work-up 1 [%]</th>
<th>Recovery, work-up 2 [%]</th>
<th>Recovery, work-up 3 [%]</th>
<th>Mean recovery [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>99.9</td>
<td>99.7</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>50</td>
<td>100.4</td>
<td>99.7</td>
<td>99.8</td>
<td>99.9</td>
</tr>
<tr>
<td>75</td>
<td>100.8</td>
<td>100.6</td>
<td>101.5</td>
<td>101.0</td>
</tr>
<tr>
<td>100</td>
<td>99.6</td>
<td>100.4</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>125</td>
<td>99.9</td>
<td>99.7</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>150</td>
<td>100.4</td>
<td>99.7</td>
<td>99.8</td>
<td>99.9</td>
</tr>
</tbody>
</table>

No. of determinations: n = 18

Overall mean recovery: 100.08%

Coefficient of variation: 0.50%

95% confidence interval: [99.83 – 100.33%] (± 0.25%)
In the case of the liquid chromatography method applied, typical variations for the robustness of the method are deliberately varying a number of parameters as follows:

- Varying the strength of the phosphoric acid used in the mobile phase

Result

Under these conditions, the best results in terms of system suitability are achieved using mobile phase prepared with 0.003 mol/L phosphoric acid. The methods which used mobile phase prepared with 0.01 mol/L and 0.001 mol/L phosphoric acid are not suitable. The order in which the components elute is not affected by the strength of the phosphoric acid.

- Varying the concentrations of the organic and aqueous components of the mobile phase

Result

Changing the concentration of the acetonitrile in the mobile phase by 1% markedly alters retention times and leads to changes in resolution. The tailing factor is not affected to the same degree as the other two parameters. The order in which the components elute is not affected by the acetonitrile concentration.

For the remaining robustness tests, the composition of the mobile phase was fixed at 12% THF / 7% ACN / 81% 0.003 mol/L phosphoric acid (10.7 g / 5.46 g / 81 g). The method remains selective with respect to the characteristic flavonoids at acetonitrile concentrations between 6 and 8%.

- Using different stationary phases

Result:

System suitability is satisfactory with regard to resolution and tailing factor irrespective of whether RP-8 or RP-18 stationary phases are used. The Symmetry Shield RP-8 column is the most suitable alternative to the Superspher column specified, although its use would necessitate changes to the HPLC conditions.

- Optimising the separation by using an alternative column (Symmetry Shield RP-8) and changing the composition of the mobile phase

Result:

The Symmetry Shield RP-8 column is the most suitable alternative to the Superspher column specified, although its use would necessitate changes to the HPLC conditions.

- Using different batches of the Superspher RP-8 (e) stationary phase

Result:

Marked variations in retention times and changes in resolution were seen with different batches of the stationary phase. The system pressure was similar in all the columns containing stationary phase with a known batch number but higher in the column containing stationary phase of unknown batch origin. There was no significant change in the tailing factor. Selectivity was maintained irrespective of the batch origin of the stationary phase and the order in
which the components eluted was not affected. The reproducibility of the method is therefore not dependent on the batch of stationary phase used.

  - Varying the flow rate

Result:
Increasing or decreasing the flow rate by 0.2 mL/min leads to variations of up to 5.5 min in the retention times, depending on the component. All three flavonoids behave similarly in this respect. There were only very minor changes in the resolution and tailing factor. The method is selective at all four flow rates. The order in which the components elute is not affected.

  - Using a column of different dimensions and changing the flow rate

Result:
Using a column with a narrower i.d. leads to variations of up to 4 min in the retention times, depending on the component. All three flavonoids behave similarly in this respect. There were only very minor changes in the resolution and tailing factor. The method is selective under both of the conditions outlined above. The order in which the components elute is not affected.

  - Varying the column oven temperature

Result:
Increasing or decreasing the temperature leads to variations of up to 6.5 min in the retention times, depending on the component. All three flavonoids behave similarly in this respect. There were only very minor changes in the tailing factor. The resolution is sensitive to changes in temperature. The method is selective at all four temperatures. The separation must therefore be carried out at a constant temperature (e.g. 25°C).

Details of results are not presented here.

Assay of anthocyanin content

Sample preparation
400 mg of powdered red vine leaf were extracted three times with 9 mL hydrochloric acid 0.25% in methanol/water 1:1 by ultrasonication. The extraction was made up to 10.0 mL with
solvent and centrifuged before use. For the reference solution malvidin-3-glucoside chloride 4 mg was dissolved in 16 mL solvent applying ultrasonication and made up to 20.0 mL.

**Apparatus and analytical conditions**
Standard HPLC equipment consisting of a binary gradient pump, column oven, solvent unit with degasser, sampler and UV/VIS detector. Comparative analysis was carried out with an injection volume of 20 µL using a LiChrospher 100RP 18 5 µm column with column temperature at 40°C. The mobile phase was a binary eluent of (A) 87% water, 10% formic acid, 3% acetonitrile and (B) 50% acetonitrile, 40% water, 10% formic acid under gradient conditions (Table below). Flow rate was 0.8 mL/min and detection wavelength was 518 nm. The content in total anthocyanins was calculated taking into account the molecular weight of each component. A typical HPLC chromatogram of anthocyanins in red vine leaves is shown in Fig. 1 in the manuscript.

Table: Gradient conditions of mobile phase for HPLC analysis of anthocyanins

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>0.0</th>
<th>15.0</th>
<th>30.0</th>
<th>35.0</th>
<th>36.0</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>% B</td>
<td>6</td>
<td>30</td>
<td>50</td>
<td>60</td>
<td>6</td>
<td>stop</td>
</tr>
</tbody>
</table>

**Specificity**
Specificity of the nine main peaks of the HPLC chromatogram was tested by their retention time and identified by reference substances. A representative chromatogram is presented in Fig 1.

**Linearity**
Oeninchloride (Malvidin-3-glucoside) used as reference standard:

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Oeninchloride [mg/mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0004</td>
<td>19.9820</td>
</tr>
<tr>
<td>2</td>
<td>0.0010</td>
<td>50.5188</td>
</tr>
<tr>
<td>3</td>
<td>0.0019</td>
<td>102.4035</td>
</tr>
<tr>
<td>4</td>
<td>0.0048</td>
<td>264.8190</td>
</tr>
<tr>
<td>5</td>
<td>0.0239</td>
<td>1’360.4188</td>
</tr>
<tr>
<td>6</td>
<td>0.0478</td>
<td>2’707.5530</td>
</tr>
</tbody>
</table>

Unweighted linear regression \( y = a + b*x \)

Number of determinations \( n = 6 \)

Slope of regression line \( b = 5.673E+04 \)
relative confidence interval (95%) +/- 0.50%
y-intercept
confidence interval (95%): a = -3.605E+00
-9.830E+00 to 2.620E+00
Relative standard deviation of method 0.58%
Correlation coefficient 0.99999

Ungewichtete lineare Regression Serie 1

Calculated from linear regression by means of residual standard deviation

Detection limit 2.540E-04 [mg/mL]
Quantitation limit 7.696E-04 [mg/mL]

Test on linearity of single anthocyanins in an extract sample

Delphinidin-3-glucoside (Del-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Del-3-gl</th>
<th>Spissum [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>204.21</td>
<td>200.0028</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>311.29</td>
<td>296.9500</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>414.70</td>
<td>388.1249</td>
</tr>
</tbody>
</table>
Unweighted linear regression \( y = a + b \times x \)

Number of determinations \( n = 3 \)

Slope of regression line \( b = 8.938 \times 10^{-1} \) relative confidence interval (95%) +/- 9.72%

y-intercept \( a = 1.789 \times 10^{1} \) confidence interval (95%): -1.006 to 4.583

Relative standard deviation of method 0.37%
Correlation coefficient 0.9997

Cyanidin-3-glucosid2 (Cy-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>427.6440</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>648.0261</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>856.8092</td>
</tr>
</tbody>
</table>

Unweighted linear regression \( y = a + b \times x \)

Number of determinations \( n = 3 \)

Slope of regression line \( b = 2.039 \times 10^{0} \) relative confidence interval (95%) +/- 7.04%

y-intercept \( a = 1.193 \times 10^{1} \)

confidence interval (95%): -3.422E+01 to 5.809E+01

Relative standard deviation of method 0.27%
Correlation coefficient 0.99998

Unweighted linear regression Serie 2

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Pt-3-gl Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>181.5862</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>287.8159</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>381.5437</td>
</tr>
</tbody>
</table>

Unweighted linear regression \( y = a + b \times x \)

Number of determinations \( n = 3 \)

Slope of regression line \( b = 9.502E-01 \)
relative confidence interval (95%) +/- 33.06%
y-intercept  
confidence interval (95%):  

\[ a = -1.098 \times 10^1 \]  
-1.121E+02 to 9.011E+01  

Relative standard deviation of method  
Correlation coefficient  

1.25%  
0.99966  

Unweighted linear regression Series 3

Peonidin-3-glucoside (Po-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>1'015.8687</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>1'529.1950</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>2'015.7592</td>
</tr>
</tbody>
</table>

Unweighted linear regression  

\[ y = a + b \times x \]  

Number of determinations  

n = 3  

Slope of regression line  

\[ b = 4.751 \times 10^0 \]  
+/- 6.84%
y-intercept: $a = 4.728E+01$
Confidence interval (95%): $-5.731E+01$ to $1.519E+02$

Relative standard deviation of method: 0.26%
Correlation coefficient: 0.99999

Malvidin-3-glucoside (Mv-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>701.5706</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>1'060.3285</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>1'402.9663</td>
</tr>
</tbody>
</table>

Unweighted linear regression: $y = a + bx$

Number of determinations: $n = 3$
Slope of regression line: $b = 3.332E+00$
relative confidence interval (95%)  

+/- 4.07%

y-intercept

confidence interval (95%):

a = 2.172E+01
-2.191E+01 to 6.534E+01

Relative standard deviation of method

0.15%

Correlation coefficient

0.99999

Ungewichtete lineare Regression Serie 5

Peonidin-3-acetyl-glucoside (Po-3-acgl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Po-3-acgl Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>33.0792</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>48.9790</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>63.7212</td>
</tr>
</tbody>
</table>

Unweighted linear regression

y = a + b*x

Number of determinations

n = 3
Slope of regression line $b = 1.456 \times 10^{-1}$

relative confidence interval (95%) $+/- 14.92\%$

$y$-intercept $a = 3.450 \times 10^{0}$

certainty interval (95%): $-3.538 \times 10^{0}$ to $1.044 \times 10^{1}$

Relative standard deviation of method 0.56%

Correlation coefficient 0.99993

Unweighted linear regression:

$$y = a + bx$$

Number of determinations $n = 3$

Malvidin-3-acetyl-glucoside (Mv-3-acgl):  

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-acgl</th>
<th>Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>33.7888</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>49.1126</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>64.5562</td>
<td></td>
</tr>
</tbody>
</table>

Unweighted linear regression $y = a + bx$

Number of determinations $n = 3$
Slope of regression line $b = 1.462E-01$
relative confidence interval (95%) $\pm 15.65\%$

y-intercept $a = 3.836E+00$
confidence interval (95%): $-3.523E+00$ to $1.119E+01$

Relative standard deviation of method $0.59\%$
Correlation coefficient $0.99992$

Ungewichtete lineare Regression Serie 7

Peonidin-3-cumaryl-glucoside (Po-3-cugl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>47.1512</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>71.5211</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>94.6996</td>
</tr>
</tbody>
</table>

Unweighted linear regression $y = a + b*x$
Number of determinations \( n = 3 \)

Slope of regression line \( b = 2.259 \times 10^{-1} \)
relative confidence interval (95%) \( +/- 5.59\% \)

y-intercept \( a = 1.079 \times 10^{0} \)
confidence interval (95%): \(-2.986 \times 10^{0}\) to \(5.143 \times 10^{0}\)

Relative standard deviation of method \( 0.21\% \)
Correlation coefficient \( 0.99999 \)

Unweighted linear regression

\[
y = a + bx
\]

Malvidin-3-cumaryl-glucoside (Mv-3-cugl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-cugl Spissum 550 [mg/10 mL]</th>
<th>Peak area [mAU * s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.21</td>
<td>94.4970</td>
</tr>
<tr>
<td>2</td>
<td>311.29</td>
<td>140.0156</td>
</tr>
<tr>
<td>3</td>
<td>414.70</td>
<td>190.8981</td>
</tr>
</tbody>
</table>

Unweighted linear regression
Number of determinations \( n = 3 \)

Slope of regression line \( b = 4.578 \times 10^{-1} \)
relative confidence interval (95%) +/- 53.63%

y-intercept \( a = -1.418 \times 10^{-1} \)
confidence interval (95%): -7.913 \times 10^1 \text{ to } 7.885 \times 10^1

Relative standard deviation of method 2.03%
Correlation coefficient 0.99911

Range

Malvidin-3-glucosid (Oeninchloride) is used as external reference. So derived from linearity study of this substance the minimum range of 80 to 120 percent of test concentration can be specified. In addition linearity of each anthocyanin in the extract matrix was tested.

Accuracy
Accuracy can be inferred because precision, linearity and specificity have been established. In addition a regression curve was established for the comparison of HPLC with the independent photometric procedure of Pharm. Franc. X (Fig. 7).

**Precision, Repeatability**

Repeatability for each of the analysed anthocyanin was calculated separately.

**Delphinidin-3-glucosid (Del-3-gl):**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Del-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00395</td>
</tr>
<tr>
<td>2</td>
<td>0.00396</td>
</tr>
<tr>
<td>3</td>
<td>0.00411</td>
</tr>
</tbody>
</table>

Number of determinations  
\( n = 3 \)

Mean  
\( 4.007 \times 10^{-3} \)  
Confidence interval (95%)  
\( 3.786 \times 10^{-3} \) to \( 4.228 \times 10^{-3} \)

Variance  
\( 7.920 \times 10^{-9} \)

Standard deviation  
\( 8.899 \times 10^{-5} \)  
Confidence interval (95%)  
\( 5.142 \times 10^{-5} \) to \( 3.929 \times 10^{-4} \)

Relative standard deviation  
(coefficient of variation)  
\( 2.22\% \)

**Cyanidin-3-glucosid (Cy-3-gl):**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Cy-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01150</td>
</tr>
<tr>
<td>2</td>
<td>0.01142</td>
</tr>
<tr>
<td>3</td>
<td>0.01194</td>
</tr>
</tbody>
</table>

Number of determinations  
\( n = 3 \)

Mean  
\( 1.162 \times 10^{-2} \)  
Confidence interval (95%)  
\( 1.092 \times 10^{-2} \) to \( 1.232 \times 10^{-2} \)

Variance  
\( 7.978 \times 10^{-8} \)

Standard deviation  
\( 2.825 \times 10^{-4} \)  
Confidence interval (95%)  
\( 1.632 \times 10^{-4} \) to \( 1.247 \times 10^{-3} \)
Relative standard deviation (coefficient of variation) 2.43%

Petunidin-3-glucosid (Pt-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Pt-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00297</td>
</tr>
<tr>
<td>2</td>
<td>0.00298</td>
</tr>
<tr>
<td>3</td>
<td>0.00310</td>
</tr>
</tbody>
</table>

Number of determinations \( n = 3 \)

Mean 3.017E-03
Confidence interval (95%) 2.843E-03 to 3.190E-03

Variance 4.883E-09
Standard deviation 6.988E-05
Confidence interval (95%) 4.037E-05 to 3.086E-04

Relative standard deviation (coefficient of variation) 2.32%

Peonidin-3-glucosid (Po-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Po-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01560</td>
</tr>
<tr>
<td>2</td>
<td>0.01558</td>
</tr>
<tr>
<td>3</td>
<td>0.01628</td>
</tr>
</tbody>
</table>

Number of determinations \( n = 3 \)

Mean 1.582E-02
Confidence interval (95%) 1.483E-02 to 1.681E-02

Variance 1.580E-07
Standard deviation 3.974E-04
Confidence interval (95%) 2.296E-04 to 1.755E-03

Relative standard deviation (coefficient of variation) 2.51%

Malvidin-3-glucosid (Mv-3-gl):
<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01098</td>
</tr>
<tr>
<td>2</td>
<td>0.01109</td>
</tr>
<tr>
<td>3</td>
<td>0.01165</td>
</tr>
</tbody>
</table>

Number of determinations \( n = 3 \)

Mean: 1.124E-02
Confidence interval (95%): 1.035E-02 to 1.213E-02

Variance: 1.280E-07
Standard deviation: 3.578E-04
Confidence interval (95%): 2.067E-04 to 1.580E-03

Relative standard deviation (coefficient of variation): 3.18%

Peonidin-3-acetyl-glucosid (Po-3-acgl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Po-3-acgl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00064</td>
</tr>
<tr>
<td>2</td>
<td>0.00064</td>
</tr>
<tr>
<td>3</td>
<td>0.00067</td>
</tr>
</tbody>
</table>

Number of determinations \( n = 3 \)

Mean: 6.526E-04
Confidence interval (95%): 6.065E-04 to 6.987E-04

Variance: 3.449E-10
Standard deviation: 1.857E-05
Confidence interval (95%): 1.073E-05 to 8.200E-05

Relative standard deviation (coefficient of variation): 2.85%

Malvidin-3-acetyl-glucosid (Mv-3-acgl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-acgl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00056</td>
</tr>
<tr>
<td>2</td>
<td>0.00058</td>
</tr>
<tr>
<td>3</td>
<td>0.00060</td>
</tr>
</tbody>
</table>
Number of determinations \( n = 3 \)

Mean \( 5.794 \times 10^{-4} \)
- Confidence interval (95%) \( 5.208 \times 10^{-4} \) to \( 6.381 \times 10^{-4} \)

Variance \( 5.579 \times 10^{-10} \)
- Standard deviation \( 2.362 \times 10^{-5} \)
  - Confidence interval (95%) \( 1.365 \times 10^{-5} \) to \( 1.043 \times 10^{-4} \)

Relative standard deviation (coefficient of variation) 4.08%

**Peonidin-3-cumaryl-glucosid (Po-3-cugl):**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Po-3-cugl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00116</td>
</tr>
<tr>
<td>2</td>
<td>0.00115</td>
</tr>
<tr>
<td>3</td>
<td>0.00122</td>
</tr>
</tbody>
</table>

Number of determinations \( n = 3 \)

Mean \( 1.176 \times 10^{-3} \)
- Confidence interval (95%) \( 1.088 \times 10^{-3} \) bis \( 1.264 \times 10^{-3} \)

Variance \( 1.258 \times 10^{-9} \)
- Standard deviation \( 3.546 \times 10^{-5} \)
  - Confidence interval (95%) \( 2.049 \times 10^{-5} \) to \( 1.566 \times 10^{-4} \)

Relative standard deviation (coefficient of variation) 3.02%

**Malvidin-3-cumaryl-glucosid (Mv-3-cugl):**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-cugl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00801</td>
</tr>
<tr>
<td>2</td>
<td>0.00798</td>
</tr>
<tr>
<td>3</td>
<td>0.00821</td>
</tr>
</tbody>
</table>

Number of determinations \( n = 3 \)

Mean \( 8.065 \times 10^{-3} \)
- Confidence interval (95%) \( 7.761 \times 10^{-3} \) to \( 8.369 \times 10^{-3} \)

Variance \( 1.498 \times 10^{-8} \)
Standard deviation 1.224E-04
Confidence interval (95%) 7.071E-05 to 5.404E-04

Relative standard deviation
(coefficient of variation) 1.52%

Precision of determination of single anthocyanins in an extract preparation

Delphinidin-3-glucosid (Del-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Del-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01462</td>
</tr>
<tr>
<td>2</td>
<td>0.01423</td>
</tr>
<tr>
<td>3</td>
<td>0.01450</td>
</tr>
</tbody>
</table>

Number of determinations n = 3
Mean 1.445E-02
Confidence interval (95%) 1.394E-02 to 1.495E-02

Variance 4.113E-08
Standard deviation 2.028E-04
Confidence interval (95%) 1.172E-04 to 8.955E-04

Relative standard deviation
(coefficient of variation) 1.40%

Cyanidin-3-glucosid (Cy-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Cy-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02971</td>
</tr>
<tr>
<td>2</td>
<td>0.03019</td>
</tr>
<tr>
<td>3</td>
<td>0.03036</td>
</tr>
</tbody>
</table>

Number of determinations n = 3
Mean 3.009E-02
Confidence interval (95%) 2.925E-02 to 3.092E-02

Variance 1.128E-07
Standard deviation 3.358E-04
Confidence interval (95%) 1.940E-04 to 1.483E-03

Relative standard deviation 1.12%
(coefficient of variation)

Petunidin-3-glucosid (Pt-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Pt-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01331</td>
</tr>
<tr>
<td>2</td>
<td>0.01326</td>
</tr>
<tr>
<td>3</td>
<td>0.01326</td>
</tr>
</tbody>
</table>

Number of determinations $n = 3$

Mean 1.328E-02
Confidence interval (95%) 1.321E-02 to 1.335E-02

Variance 7.821E-10
Standard deviation 2.797E-05
Confidence interval (95%) 1.616E-05 to 1.235E-04

Relative standard deviation (coefficient of variation) 0.21%

Peonidin-3-glucosid (Po-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Po-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07048</td>
</tr>
<tr>
<td>2</td>
<td>0.07049</td>
</tr>
<tr>
<td>3</td>
<td>0.07082</td>
</tr>
</tbody>
</table>

Number of determinations $n = 3$

Mean 7.060E-02
Confidence interval (95%) 7.012E-02 to 7.107E-02

Variance 3.697E-08
Standard deviation 1.923E-04
Confidence interval (95%) 1.111E-04 to 8.490E-04

Relative standard deviation (coefficient of variation) 0.27%

Malvidin-3-glucosid (Mv-3-gl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-gl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Nr.</td>
<td>Po-3-acgl [%]</td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>0.00249</td>
</tr>
<tr>
<td>2</td>
<td>0.00252</td>
</tr>
<tr>
<td>3</td>
<td>0.00250</td>
</tr>
</tbody>
</table>

Number of determinations: \( n = 3 \)

Mean: \( 5.142 \times 10^{-2} \)
Confidence interval (95%): \( 5.108 \times 10^{-2} \) to \( 5.175 \times 10^{-2} \)

Variance: \( 1.778 \times 10^{-8} \)
Standard deviation: \( 1.333 \times 10^{-4} \)
Confidence interval (95%): \( 7.703 \times 10^{-5} \) to \( 5.887 \times 10^{-4} \)

Relative standard deviation (coefficient of variation): 0.26%

Malvidin-3-acetyl-glucosid (Mv-3-acgl):
Mean 2.851E-03
Confidence interval (95%) 2.836E-03 to 2.867E-03

Variance 3.749E-11
Standard deviation 6.123E-06
Confidence interval (95%) 3.537E-06 to 2.703E-05

Relative standard deviation (coefficient of variation) 0.21%

Peonidin-3-cumaryl-glucosid (Po-3-cugl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Po-3-cugl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00445</td>
</tr>
<tr>
<td>2</td>
<td>0.00447</td>
</tr>
<tr>
<td>3</td>
<td>0.00444</td>
</tr>
</tbody>
</table>

Number of determinations n = 3

Mean 4.453E-03
Confidence interval (95%) 4.409E-03 to 4.497E-03

Variance 3.198E-10
Standard deviation 1.788E-05
Confidence interval (95%) 1.033E-05 to 7.896E-05

Relative standard deviation (coefficient of variation) 0.40%

Malvidin-3-cumaryl-glucosid (Mv-3-cugl):

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mv-3-cugl [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00919</td>
</tr>
<tr>
<td>2</td>
<td>0.00914</td>
</tr>
<tr>
<td>3</td>
<td>0.00920</td>
</tr>
</tbody>
</table>

Number of determinations n = 3

Mean 9.173E-03
Confidence interval (95%) 9.094E-03 to 9.251E-03
Assay of procyanidin content

The qualitative and quantitative determination of this group of substances is hampered by the difficulty to separate the single compounds and by the lack of reference standards. Our approach was therefore to concentrate on the spectrum of substances and a semi-quantitative determination. Therefore procyanidins are not included in the specification for red vine leaves.

Sample preparation

100 mg of an aqueous extract of red vine leaves were dissolved in sodium acetate buffer pH 5.3 by applying ultrasonication and filtered. The reference substances were dissolved in sodium acetate buffer pH 5.3/acetonitrile 5:1 with substance concentrations between 0.5 and 3.5 mg/100 mL.

Apparatus and analytical conditions

HPLC equipment consisting of a binary HPLC pump with column oven, autosampler and UV/VIS diode array detector. The semi-quantitative assay was carried out with an injection volume of 20 µL using a Spherisorb S5 ODS-B 5µm column at a temperature of 30°C. The mobile phase was a binary eluent of (A) acetic acid 2.5% and (B) acetonitrile /mobile phase A 60/40 with a gradient of mobile phase (B) from 0 to 10% in 30 min, to 16 % after 40 min to 26% after 62 min and 90 after 75 min. The flow rate was 1 mL/min and detection wavelength was 280 nm. A typical chromatogram is shown in Fig. 3 in the manuscript.

Specificity

The use of reference substances allowed to identify some of the peaks of the HPLC chromatogram.

A typical chromatogram is shown in Fig. 4

Validation of analytical methods
The purpose of our investigation on procyanidins was to get an overview of the components and their order of magnitude in red vine leaves by semi-quantitative evaluation. The method was therefore not validated for assay purposes.