antioxidants

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Supplementary Materials: Healthberry 865® and Its Related, Specific, Single Anthocyanins Exert a Direct Vascular Action, Modulating Both Endothelial Function and Oxidative Stress

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Anthocyanins quantification by HPLC-MS method

By using an HPLC-MS method it was possible to detect and quantify the present anthocyanins in Healthberry 865. This was done due to external calibration by using single anthocyanins as reference material and therefore being more specific and precise. As their purity was unknown, every single anthocyanin was analyzed by HPLC-MS prior to the analysis.

All the reference materials contained trace amounts resulting in little peak responses. DP3-rut contained DP3-glu and could therefore not be used for direct quantification. In order to get a result, the amounts of DP3-glu and DP3-rut were estimated by their areas: area of the peaks formed by DP3-glu and DP3-rut = 100 %. DP3-ara could not be detected in the reference material but in Healthberry 865.

Table 1 presents the results for the quantitation of all relevant anthocyanins. Figure 1 presents the findings as graphical summary.

Anthocyanin	Healthberry HPLC MS [% w/w]
DP3-gal	1.65
C3-gal	1.21
C3-glu	1.57
C3-arapy	2.37
C3-rut	3.19
PT3-glu	2.03
PEO3-gal	0.20
PEO3-glu	0.96
MAL3-gal	0.16
MAL3-glu	5.13
DP3-glu	-
DP3-ara	-
DP3-rut	3.18
PT3-gal	0.48

PEO3-ara	0.61
Sum HPLC	22.74
Sum UV	39.07

Table S1. Results for the quantitation of all relevant anthocyanins.

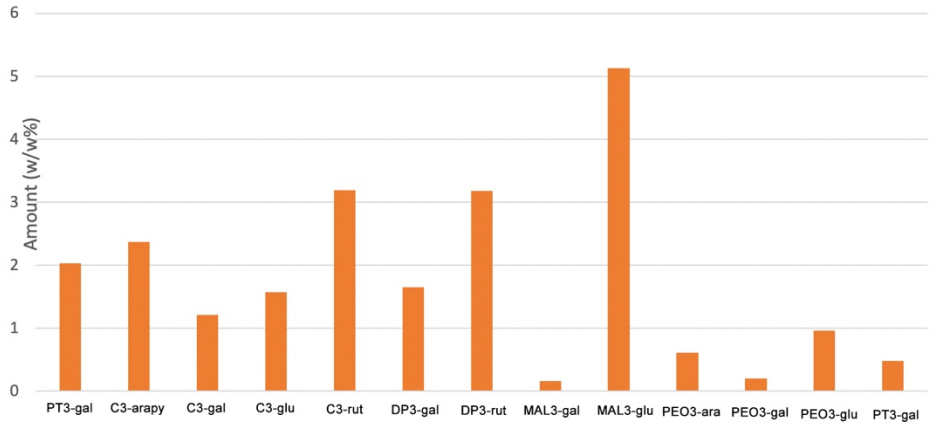

Figure S1. HPLC analysis of Healthberry 865.

Method and Results.

Analytical Method	**HPLC-UV/MS**
Instrument	Accela 1250 equipped with an Orbitrap Elite (Thermo Fischer)
Detection	ESI positive, UV (520 nm)
Mass Range	400 – 650 u
Analytical Column	250x4 mm EC NUCLEODUR π2, 5 µm (Macherey-Nagel, REF 760625.40)
Eluent A	H2O + Formic Acid (94/6, v/v)
Eluent B	Methanol
Gradient	5%B – in 30 min - 95% - 10 min isocratic
Posttime	10 min
Flow	0.75 mL/min
Temperature	65°C
Injection volume	1 µL
Diluent	H2O + Formic Acid (94/6, v/v)
Sample Preparation	approx. 1 mg/mL in Diluent

The method was not developed by the laboratory itself. A commercial analytic column for detection of anthocyanins was used, provided by the manufacture: MACHEREY-NAGEL GmbH & Co. KG, Neumann-Neander-Str. 6–8, 52355 Düren/D.

Literatur: "Bestimmung von Anthocyanen in Fruchtsäften per HPLC – UV", H. R. Wollseifen, Düren/D, T. Kretschmer; http://ftp.mnnet.com/deutsch/Poster/Chromatographie/Poster_Anthocyane_Fruchtsaefte_www.pdf (12/18/2019,14:38 (MEZ))

However, the amount of formic acid in the eluent system was increased to increase separation of isomeric compounds. The resulting chromatograms of the analysis of the sample materials Healtberry 865 are shown in figure 2 to 3. As the purity and/or the

content of most of the reference materials was unknown, each reference material was analyzed by HPLC-MS.

All the reference materials contain trace amounts resulting in little peak responses. As the content of the reference materials is unknown, there is already a certain error in the quantitation results. An analytical method itself also provides a certain error. Therefore, the little peak responses could be neglected in this case.

DP3-rut contained DP3-glu. As the calibration solutions contains all the reference materials the amount of DP3-glu and the amount of DP3-rut in the calibration solutions are unknown. Therefore, the calibration of these two anthocyanoses was not possible based on the current data.

A different sample of DP3-rut also contained a certain, unknown amount of DP3-glu. Therefore, the calibration of DP3-rut was not possible based on the current data. In order to get a result, the amounts of DP3-glu and DP3-rut were estimated by their areas: area of the peaks formed by DP3-glu and DP3-rut = 100 %. This basic calculation only provides a rough estimation, as the response factors of these two molecules are unknown.

DP3-ara could not be detected in the reference material but in the sample materials Healthberry 865.

The quantitation of the anthocyanins in Healthberry 865 by HPLC-MS was accomplished by an external calibration with the given reference materials. As the purity and/or the content of most of these reference materials is unknown, the quantitation results for these compounds are based on an assumed content of 100 %.

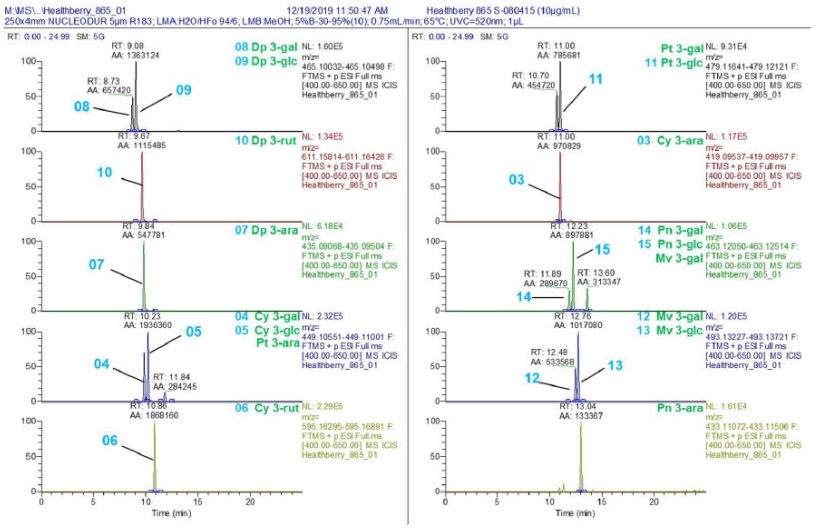

Figure S2. EICS (extracted ion chromatograms), covering all relevant anthocyanins, part 1.

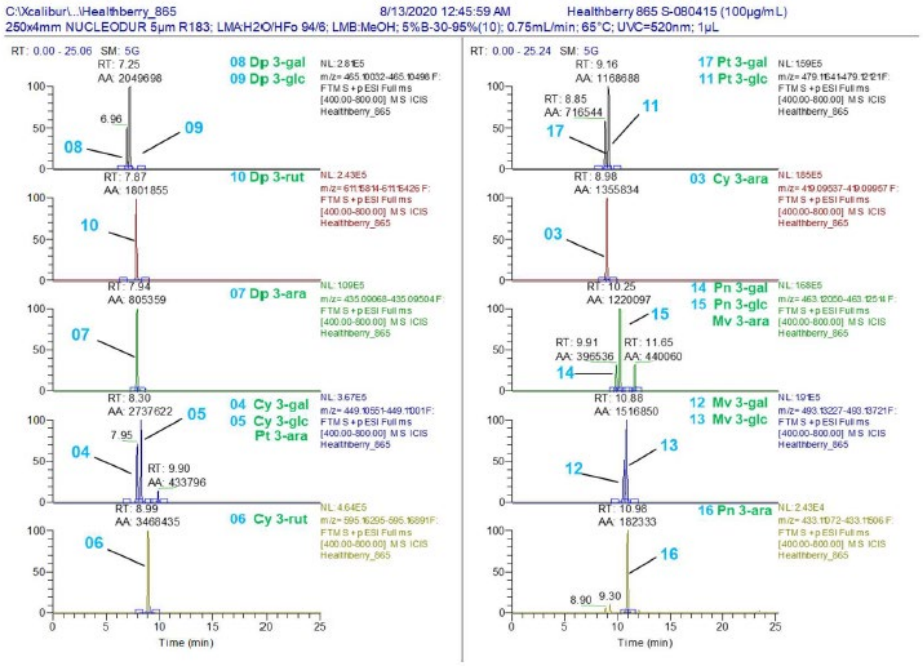

Figure S3. EICS (extracted ion chromatograms), covering all relevant anthocyanins, part 2.

List of supplied anthocyanins.

No.	Product Description	Provider
1	Healthberry 865	Evonik Industries
3	C3-gal	Polyphenols
4	C3-glu	Polyphenols
5	C3-rut	Polyphenols
6	DP3-arapy	AOBIOUS
7	DP3-gal	Sigma Aldrich
8	DP3-glu	Polyphenols
9	DP3-rut	Polyphenols
10	PT3-glu	Polyphenols
11	MAL3-gal	Sigma Aldrich
12	MAL3-glu	Polyphenols
13	PEO3-gal	Polyphenols

Clinical characteristics	Subjects (N=4)
Age average, y	56,1 ± 4
Risk factors, n (%)	
Hypertension	100%
Dyslipidemia	0
Cardiopathy	0
Diabetes mellitus	0
Smoker	25%
COPD	0
Hepatopathy	0
Dysthyroidism	0

Previous surgery	25%
Medications, n (%)	
Diuretics	100%
ASA	0
ACE inhibitors	0
Statins	0

Table S2. Clinical characteristics of patients.