Validation of HPLC-PDA method for determination of polyphenol profile of Croatian traditional apple cultivars

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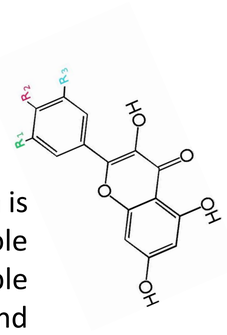
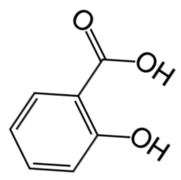
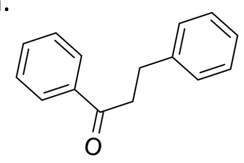
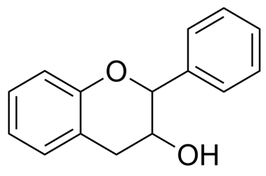
Introduction



Apple fruit is a major source of phenol compounds, because its consumption is widespread in many countries and it is available on the market for the whole year. Generally, five major polyphenolic groups are found in various apple varities: hydroxycinnamic acids, flavan-3-ols, anthocyanins, flavonols and dihydrochalcones. Polyphenols are molecules with strong antioxidant activity that plants synthesize in response to stress conditions. Apple cultivars containing higher levels of procyanidins, polyphenols, dihydrochalcones, flavan-3-ols, flavanols and phenolic acids, are more resistant to *P.expansum* which causes blue mould. Furthermore, apple polyphenols are involved in the response to patulin contamination, as they neutralize the free radicals induced by patulin.

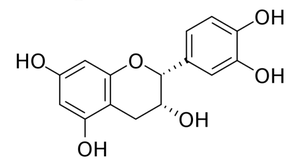






Materials & Methods

HPLC method was established, optimized and validate for the separation and quantitation of 19 polyphenols extracted from the Croatian traditional apple cultivars. As stationary phase was used a reversed- phase C18 column, and an acidified water and methanol were used as mobile phase. The injection volume of the sample was 10 µL, the flow of the mobile phase was set to 0,8 mL/min, and the temperature of the column and detector to 50°C. The polyphenols were detected using photo-diode array detector (PDA) at 280, 320, 360 and 520 nm. For the evaluation of fitness for purpose, linearity, trueness and precision were determined and all validation parameters were acceptable for all determined polyphenols. To prepare the apple extract, 500 mg of apple trop was weighed and dissolved in 2,5 mL of 80% methanol.



Results

Identification of the separated components was performed based on the retention time and comparison of the absorption spectra of the components in the apple extract with the spectra of the standards, while the quantification of the components was performed based on the external calibration method.

Table 1: Detection wavelengths and correlation coefficients (r)

Component	λ	r
IDC	520	0,9999633
GA	280	0,9999866
FA	320	0,9999834
CHA	320	0,9999809
EGC	280	0,9991971
CA	320	0,9995392
PA2	280	0,9999338
CAT	280	0,9998230
pCA	320	0,9999413
PHL	280	0,9998760
EPI	280	0,9993037
MY	360	0,9997800
RU	360	0,9997720
Q3G	360	0,9999349
PB1	280	0,9951555
ODC	520	0,9995805
QUE	360	0,9995243
PB2	280	0,9997888
P3G	520	0,9992155

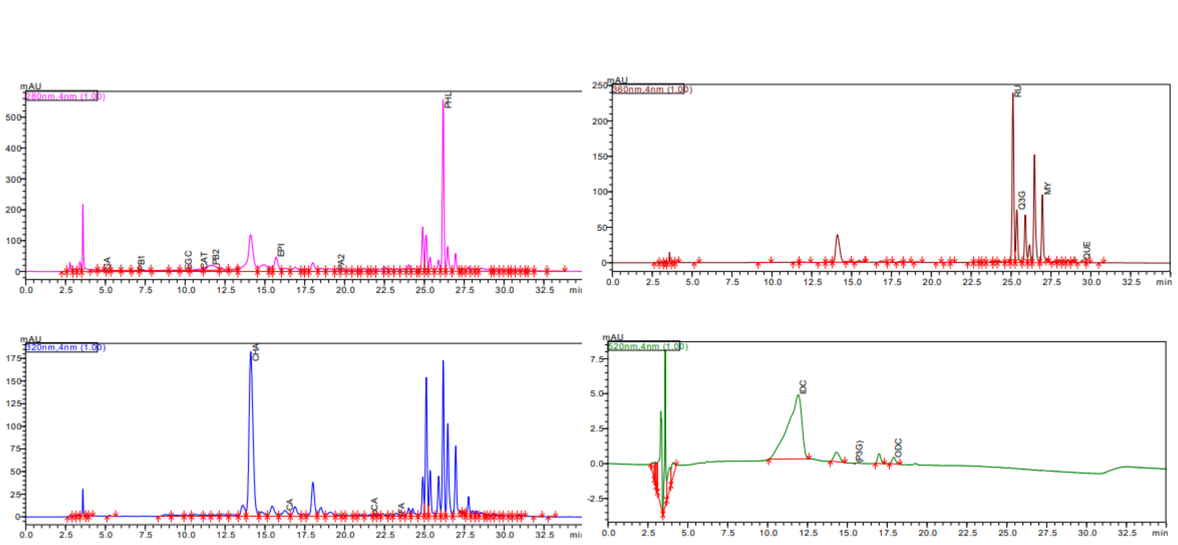
Table 2: The truth of the method

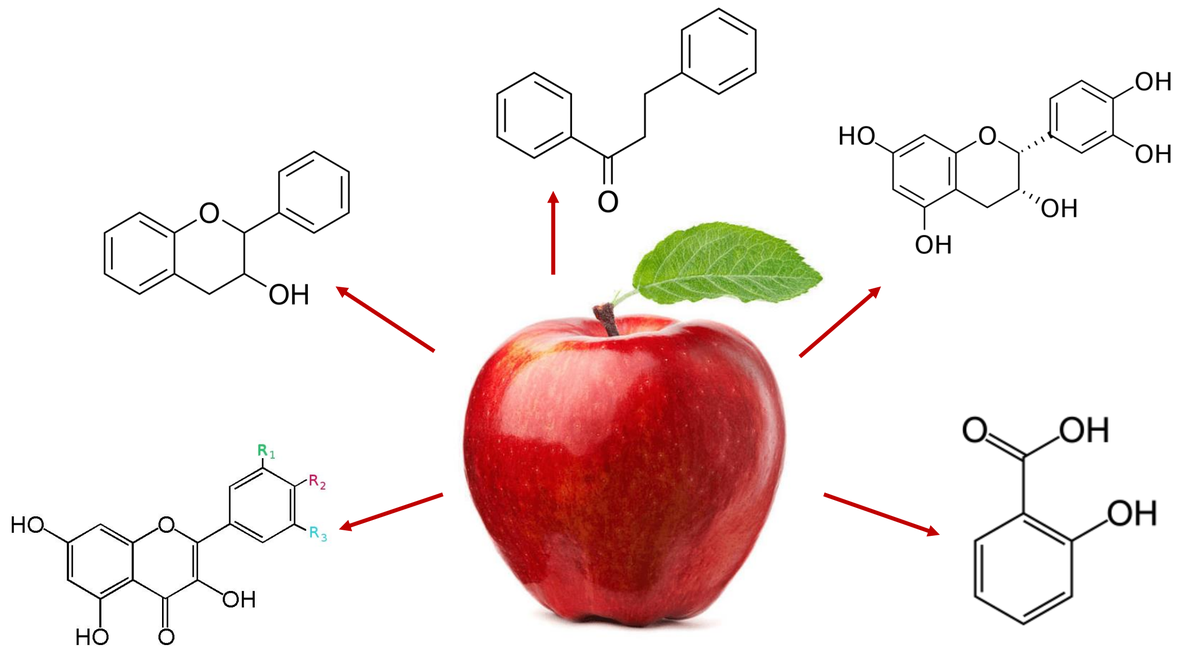
Measured value (µg/mL)	Expected value (µg/mL)	Utilization%
11,83	12,85	92,0
10,52	10,62	99,0
10,76	11,06	97,3
65,26	64,11	101,8
15,86	15,28	103,8
11,49	11,35	101,2
15,34	15,28	100,4
15,55	16,15	96,3
11,48	10,66	107,7
77,69	74,18	104,7
46,56	47,40	98,2
16,76	16,78	99,9
40,90	40,35	101,4
20,09	20,19	99,5
15,61	15,49	100,7
8,74	9,77	89,5
11,46	10,49	109,2
95,40	96,59	98,8
9,59	10,18	94,2

Table 3: Repeatability of solution preparation and repeatability of measurements.

Component	Mean value	Standard deviations	Mean Value	Standard deviations
IDC (µg/g)	113,45	7,45	103,96	1,13
GA (µg/g)	0,00	0,00	0,15	0,11
FA (µg/g)	4,13	0,63	3,63	0,60
CHA (µg/g)	530,29	12,04	516,43	1,69
EGC (µg/g)	18,59	4,20	20,83	4,09
CA (µg/g)	8,22	0,54	7,77	0,54
PA2 (µg/g)	5,09	0,61	6,05	0,76
CAT (µg/g)	46,47	1,43	46,73	1,60
pCA(µg/g)	1,41	0,42	1,08	0,40
PHL (µg/g)	611,66	21,13	577,98	1,81
EPI (µg/g)	309,45	6,93	299,32	2,30
MY (µg/g)	62,85	3,46	56,60	2,35
RU (µg/g)	300,50	14,02	278,62	1,31
Q3G (µg/g)	104,72	4,46	97,89	0,40
PB1 (µg/g)	52,58	1,91	53,83	2,11
ODC (µg/g)	1,30	0,05	1,19	0,04
QUE (µg/g)	3,10	0,10	3,01	0,08
PB2 (µg/g)	841,42	18,35	816,96	12,28
P3G (µg/g)	0,00	0,00	0,00	0,00

GA- gallic acid; CA-coffee acid; FA- trans-ferulic acids; CHA- chlorogenic acid; pCA- p-coumaric acid; RU-routine; QUE-quercetin; Q3G- quercetin-3-ß-D-glucoside; MY-myricetin; PHL- floridzina; EPI- (-)-epicatechin; CAT- (+)-catechin; EGC-epigalocatechin; PB1- procyanidin B1; PB2- procyanidin B2; PA2- procyanidin A2; IDC- idaei chloride; ODC- oenine chloride; P3G- pelargonidine-3-glucoside





Conclusion

The method was successfully applied for simultaneous analysis of procyanidins, dihydrochalcones, flavan-3-ols, flavonols, and phenolic acids from Croatian traditional apple cultivars. From the results shown in the Table 1.-3. , and taking into account the recommendations for the performance criteria of chromatographic methods, it can be concluded that the proposed HPLC method for the determination of polyphenols in apples is suitable for the purpose.

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