DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF FLAVONOIDS IN HERBAL PREPARATIONS

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INTRODUCTION AND AIM

Flavonoids are a large group of polyphenolic components possessing benzo-γ-pyronic structure and widely distributed in plants. The chemical nature and the biological activity of flavonoids depends on the structural class to which they belong, the degree of hydroxylation, the degree of polymerization, and the presence of other substituents and bonds. Today, there are a large number of herbal preparations containing plant extracts rich in flavonoids in the pharmacies across Republic of Macedonia, and therefore it is necessary to develop methods for controlling and monitoring their quality.

The aim of this work was to develop and validate HPLC method for determination of rutin and quercetin in tablets containing 1200 mg dry leaf of *Ginkgo biloba*

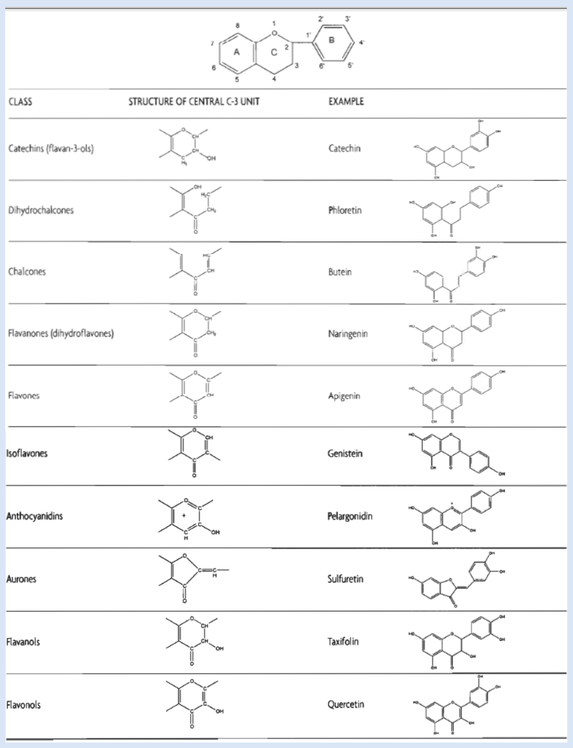

Figure 1. Chemical structure of different classes of flavonoids

MATERIALS AND METHODS

Simple HPLC method with gradient elution (acetonitrile: 0.3% phosphoric acid), flow rate of 1,2 ml/min, column temperature of 25°C and UV detection (rutin at 255nm, quercetin at 375 nm) of rutin and quercetin in tablets for oral use containing 1200 mg dry leaf of *Ginkgo biloba*.

Table 1. Chromatographic conditions of the method

Column	Purospher® STAR RP-18e (250 mm x 4,0 mm I.D., 5 µm)		
Mobile phase	Acetonitrile : 0,3% phosphoric acid		
Pump	Gradient elution		
Gradient	Time (min)	Acetonitrile (*V*, %)	0,3% phosphoric acid (*V*, %)
	0,0	15	85
	15,0	15	85
	30,0	25	75
	32,0	15	85
	35,0	15	85
Flow rate	1,2 ml/ min		
Temperature of column	25 °C		
Detection	UV, 255 nm (rutin); 375 nm (quercetin)		
Volume of sample	20 µl		

METHOD VALIDATION

Determination of:

- specificity - comparing the retention time of rutin and quercetin in standard solutions and retention time of rutin and quercetin in the sample solution.
- linearity - determining the correlation coefficient (R^2)
- precision - determined by six repetitions of the analysis of standard solution of rutin and quercetin (0.05 mg/ml)

RESULTS AND DISCUSSION

- The retention time (RT) of rutin was 14.23 minutes, while of quercetin was 29.19 minutes.
- One tablet containing 1200 mg dry leaf of *Ginkgo biloba* contains 0,479337 mg rutin and 0,00265 mg quercetin.

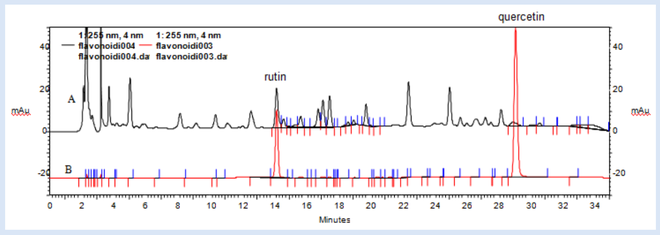

Figure 2. Chromatogram of sample solution (A) and standard solution of rutin and quercetin (B) at 255 nm

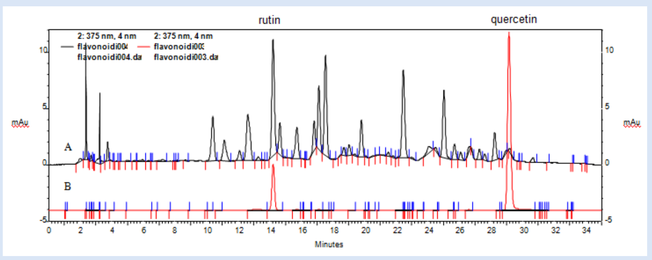

Figure 3. Chromatogram of sample solution (A) and standard solution of rutin and quercetin (B) at 375 nm

- Correlation coefficients (R^2): 1 for rutin, 0.999 for quercetin.

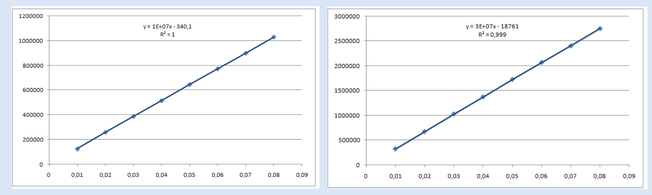

Figure 4. Linearity and correlation coefficient of calibration curve for rutin (left) and quercetin (right)

- The percentage of relative standard deviation for all parameters was less than 1%.

Table 2. Results from determination of the precision of the method

Concentration 0,05 mg/ml	Rutin (255 nm) Rt (min)	Rutin (255 nm) Area	Quercetin (375 nm) Rt (min)	Quercetin (375 nm) Area
sample 1	14,50	644571,00	29,42	1729716,00
sample 2	14,62	642136,00	29,60	1713401,00
sample 3	14,68	638141,00	29,60	1692723,00
sample 4	14,68	631984,00	29,59	1686735,00
sample 5	14,69	632369,00	29,60	1693515,00
sample 6	14,68	634781,00	29,59	1699250,00
Average	14,64	637330,33	29,57	1702556,67
StDev	0,07	5212,99	0,07	16093,11
Relative StDev (%)	0,50	0,82	0,24	0,95

CONCLUSION

This HPLC method is simple, easy to perform and specific for determination of rutin and quercetin in herbal preparations containing dry leaf of *Ginkgo biloba* and can be used for routine analysis.

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