*Short communication*

# Spectrofluorimetric and HPLC Determination of Morin in Human Serum

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## Abstract

Morin is a flavonol antioxidant. In ethanol – water mixtures (70 wt % of ethanol) it reacts with $Al^{3+}$ to give $Al(Morin)_2$ in the pH range 3–6. The conditional stability constant of this complex at 298 K was found to be $\log \beta_2 = 16.96 \pm 0.02$ at pH 4.40. The complex shows strong fluorescence emission at 500 nm upon excitation at 410 nm. The fluorescence intensity is pH dependent with maximum emission at pH 4.40. Since the complexation reaction enhances the fluorescence of morin, this property was used for the determination of morin in human serum. A linear dependence of the intensity of fluorescence of the complex on the concentration of morin was obtained in morin concentration range from 1.5–30.5 ng $mL^{-1}$, relative standard error of measurements was 1.4 %. The LOD was 0.02 ng $mL^{-1}$ while LOQ was 1.0 ng $mL^{-1}$. Serum concentration of morin was also determined using HPLC as a reference method. A C-18 Hypersil Gold AQ column was used with acetonitrile – 0.1% v/v phosphoric acid (30:70% v/v) as the mobile phase at 1.0 mL $min^{-1}$ flow rate and UV detection at 250 nm. Acceptable relative standard errors (less than 5%) between determinations obtained by the two methods indicate that the fluorescence method is reliable.

**Keywords:** Morin, aluminium, determination, spectrofluorimetry, serum

## 1. Introduction

Flavonoids are a large class of compounds consisted of A and C rings of benzo-1-pyran-4-quinone and a B ring, and further subclassified as flavones (basic structure), flavonols (having a hydroxyl group at the 3-position), isoflavones (B ring binds to the 3-position), flavanones (2–3 bond is saturated), and catechins (C-ring is 1-pyran), chalcones (C-ring is opened), and anthocyanidins (C-ring is 1-pyran, and 1–2 and 3–4 bonds are unsaturated). They may have a number of substituents such as hydroxyl and/or methoxyl groups.

Flavones can prevent many diseases including cancers through antioxidative action and/or the modulation of several enzyme functions. For example, they may reduce coronary heart disease mortality[1] by suppressing the oxidation of low-density lipoprotein.[2] Anticarcinogenic activity of flavones is expressed by their agonism and/or antagonism of carcinogenesis-related receptors such as epidermal growth factor,[3] arylhydrocarbon receptor[4] and estrogen receptor $\beta$.[5] The secretion of cytokines,[6–9] and expression of protein kinases in tumor cell proliferation[10, 11] could be modulated by polyphenols. Morin is typical representative of flavonoids which bears most of their

physiological functions. Because of that supplemental formulations containing morin are under investigations. Thus, it is of interest to develop simple, accurate and precise method for the determination of morin in human serum. Up to now, analysis of flavonoids has been accomplished by thin-layer chromatography,[12, 13] gas chromatography,[14, 15] capillary electrophoresis,[16–21] electrochemical measurements,[22–24] high-performance liquid chromatography (HPLC).[12, 25–29] Especially, HPLC was widely used to separate and analyse flavonoids.

Morin can selectively form highly colored and fluorescent complexes with aluminium, and has long been used for analysis of aluminium. Morin is weakly fluorescent by itself but forms highly fluorescent complex with aluminium.[30–32] Hollman et al.[33] applied aluminium nitrate as a post-column reagent in RP-HPLC with fluorescence detection to determine flavonols including quercetin, morin, and the like, in biological fluids. The comparative studies showed that the aluminium –morin complex had the strongest fluorescence intensity. Thus we thought to utilize the complexation reaction between aluminium and morin for the determination of morin in human plasma based on highly intensive fluorescence of aluminium-morin complex. As a reference method we used modified RP-HPLC determination of morin based on extraction of morin from serum samples with ether – acetone mixture. [34]

## 2. Experimental

### 2. 1. Reagents and Solutions

Aluminium-nitrate, morin (Fluka AG), ethanol, NaOH, $CH_3COOH$ (Merck) all p.a. grade, have been used. All reagents were used without further purification.

The stock solution of aluminium-nitrate was prepared by dissolving aluminium-nitrate in doubly distilled water. The content of Al was determined gravimetrically by precipitation with ammonia. The solution of morin was prepared by dissolving precisely measured mass of morin in 70 wt % of ethanol. This solution was stored in refrigerator.

Working solutions have been prepared by dilution of $1.0 \times 10^{-4}$ mol $L^{-1}$ $Al(NO_3)_3$ and $1.0 \times 10^{-4}$ mol $L^{-1}$ morin respectivelly.

Human pool serum was obtained from Department of Transfusion of the clinical hospital "Dr Dragisa Misovic", Belgrade, Serbia.

All measurements were made in acetate buffers (in 70 wt % ethanol) which had been prepared according to Perrin.[35]

### 2. 2. Instruments

Fluorescence spectra were collected using a Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. Samples were placed in a 1-cm optical path length quartz cuvette for spectral recording. The slits on the excitation and emission beams were fixed at 4 and 3 nm, respectively. The spectra were corrected for the dark counts. In each measurement, three scans with one- second-integration time, were averaged. The emission spectrum of the solvent (ethanol) was subtracted. All measurements were performed at 24 °C controlled by a Peltier element. Measurements of pH were caried out using pH-meter Metler Toledo mp 120 (accuracy of ±0,01 pH unit) and combined electrode.

The HPLC apparatus (Shimadzu, Japan) included quaternary pump, LC-20AT, degasser, DGU-$20A_3$, injector 7125 (20 μL), column thermostat CTO-20A and uv diode array SPD-M20A. Acquisition and data analysis were performed with manufacturer software LC Solution. The RP-18 column was Thermo-Fisher (USA) Hypersil Gold AQ (150 × 4.6 mm, 5.0 μm). Mobile phase consisted of acetonitrile/0.1% phosphoric acid at 30:70 v/v % ratio with a flow rate 1 mL $min^{-1}$ and injected volume 20 μL. Wavelength of detection was 250 nm.

## 3. Results and Discussion

### 3. 1. Complex Formation Between Morin and Aluminium(III) -ion

Morin and aluminium(III)-ion upon reaction in ethanolic solution form the yellow orange complex in the pH range 3.0–6.0. The fluorescence spectra were recorded using ethanol as a blank and excitation and emission wavelenghts maxima were $\lambda_{ex}$ = 410 nm and $\lambda_{em}$ = 500 nm, respectively.

The fluorescence of the morin solution (c = $2.0 \times 10^{-7}$ mol $L^{-1}$) incereases upon addition of $Al^{3+}$ ion. This is expected since $Al^{3+}$ forms a fluorescent complex (es)

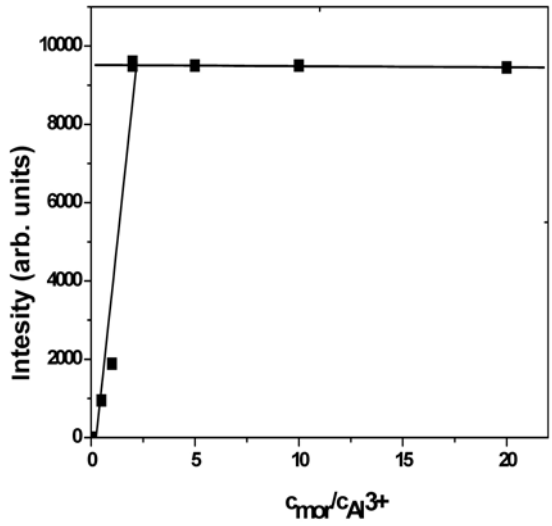


**Fig. 1:** Method of molar ratios. Dependence of intensity of fluorescence on molar ratio $c_{mor}/c_{Al3+}$

with morin of general formula $Al(morin)_n$. The response is linear from $1.0 \times 10^{-8}$ to $2.0 \times 10^{-6}$ mol $L^{-1}$ $Al^{3+}$. Above the ~s $5.0 \times 10^{-6}$ mol $L^{-1}$ $Al^{3+}$ the response levels off due to saturation of all binding sites in morin. The same type of response is obtained for $Al^{3+}$ solution with the addition of varying concentrations of morin. The stoichiometry of the complexation was investigated by using Job[36] and molar ratio[37] methods. At pH 4.40 the most probable stoichiometry is $n = 2$. The composition of aluminium –morin complex was also estimated by the mole ratio method. The result is shown in Fig. 1 which confirms the aluminium to morin ratio 1:2 for the complex formed at pH 4.40. This indicates that complexation equilibria are not stepwise and the complex is formed in a single step.

The stability constant of the complex at pH 4.40 was estimated from Jobb's plot and (conditional) stability constant was found to be log K = 16.96 ± 0.02. To examine the dependence of fluorescence intensity on pH the measurements were made in acetate buffers (in 70 wt % ethanol) of different pH values, prepared according to Perrin.[35] By subtracting relevant intensity of fluorescence of the Al $(NO_3)_3$ and morin solutions from their mixture, the curve I = f (pH) was obtained (Fig. 2).

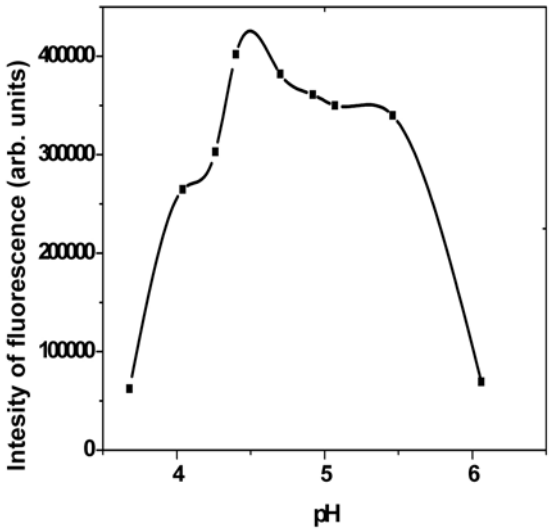


**Fig. 2:** Dependence of intensity of fluorescence on the pH

The pH dependence of fluorescence intensity exhibits a complex shape. At low pH intensity decreases because protons tend to displace $Al^{3+}$. At pH higher ~s 5.0 intensity again falls off because more of the $Al^{3+}$ is in the form of hydroxide complexes.

## 3. 2. The Quantitative Determination of Morin

The formation of a stable aluminium-morin complex in ethanolic solution with enhanced fluorescence can be utilized for quantitative determination of morin in various matrices in trace amounts. We chosed to develop and validate a method for the determination of morin in human serum.

### 3. 2. 1. Calibration Graph in Aqueous – Ethanolic Phase

***Linearity***

The high value of the stability constant of the aluminium – morin complex ensures the quantitative determination of morin using the complex. The calibration curve method was used, requiring solutions containing constant concentration of $Al(NO_3)_3$ and different concentrations of morin in acetate buffer (using 70 wt % ethanol as solvent) with pH 4.40. Blank was acetate buffer at pH 4.40. Linear dependence of the intensity of fluorescence of the complex on the concentration of morin was obtained in the interval 1.5–30.5 ng $mL^{-1}$. The regression equation:

$$I = (3.19 \pm 0.07)\, c + (0.81 \pm 0.04) \quad (1)$$

was calculated, where $I$ is fluorescence intensity in % ($\lambda_{em}$ = 500 nm) and $c$ is concentration in ng $mL^{-1}$. The good linearity of calibration curve and negligible scatter of experimental points are representing by the high correlation coefficient, r = 0.99874.

***LOD (Limit of Detection) and LOQ (Limit of Quantification)***

The limit of detection (LOD) [38, 39] was calculated by establishing the minimum level at which morin can be detected, according to formula

$$LOD = 3.3\, s_a/a \quad (2)$$

where $s_a$ is standard deviation in intercept and a is a slope of calibration line. It was found that LOD in aqueous-ethanolic solution is 0.015 ng $mL^{-1}$.

The limit of quantification (LOQ)[38, 39] was determined by using the formula:

$$LOQ = 10\, s_a/a \quad (3)$$

Morin can be quantified at a concentration of 0.045 ng $mL^{-1}$ in aqueous – ethanolic solutions.

***Precision***

The accuracy of the method was determined for three different morin concentrations (Table 1). The repeatability of the method is fairly high as indicated by low values of SD. The results obtained by proposed procedure indicate that the method is precise for the determination of morin in ethanolic media. The results are shown in Table 1.

**Table 1:** The spectrofluorimetric determination of morin in aqueous – ethanolic solutions

| Taken (ng mL$^{-1}$) | Found (mg mL$^{-1}$) | Recovery(%) | SD | CV(%) |
|---|---|---|---|---|
| 3.03 | 2.95 | 97.4 | $2.5 \times 10^{-2}$ | 0.84 |
| 6.06 | 6.08 | 100.4 | $2.9 \times 10^{-2}$ | 0.47 |
| 9.09 | 9.14 | 100.6 | $2.8 \times 10^{-2}$ | 0.31 |

n = 3

**Table 2:** Accuracy and precision of the spectrofluorimetric determination of morin in serum samples

| Taken (μg mL$^{-1}$) | Found (μg mL$^{-1}$) | Recovery(%) | SD | CV(%) |
|---|---|---|---|---|
| 0.1515 | 0.1515 | 100.00 | $2.1 \times 10^{-3}$ | 1. 40 |
| 0.3030 | 0.3036 | 100.15 | $3.5 \times 10^{-3}$ | 1.15 |
| 0.4545 | 0.4551 | 100.11 | $3.5 \times 10^{-3}$ | 0.77 |

n = 3

### 3. 2. 2. Determination of Morin in Human Serum

*Calibration graph and procedure for human serum*

A 0.2 mL human pool serum and 8.8 mL acetate buffer (in 70 wt % ethanol) pH 4.40, were mixed with standard solution of morin to give concentrations 1.5–30.5 ng mL$^{-1}$ of morin (total volume 10 mL). After incubation, a 0.5 mL of $1 \times 10^{-6}$ mol L$^{-1}$ $Al(NO_3)_3$ was added. Fluorescence spectra of prepared solutions were taken at $\lambda_{ex}$ = 410 nm and $\lambda_{em}$ = 500 nm, against the serum in acetate buffer (pH 4.40). Linear dependence of the intensity of fluorescence of the complex on the concentration of morin in diluted serum samples was obtained in the interval 1.5–30.5 ng mL$^{-1}$. The regression equation:

$$I = (3.30 \pm 0.05)\, c + (0.76 \pm 0.03)\quad r = 0.99944 \quad (4)$$

was calculated, where *I* is fluorescence intensity in %, and *c* is concentration of morin in ng mL$^{-1}$. The limit of detection (LOD) of morin in serum was calculated and it was 0.02 ng mL$^{-1}$. The limit of quantification (LOQ) of morin in serum was found to be 0.06 ng mL$^{-1}$.

To assay the accuracy of the method tree different concentrations of morin were added to a human serum in order to get its concentrations of morin in the interval 0.152–0.455 μg mL$^{-1}$. These serum samples were treated in the same way as for the calibration graph. After addition of $Al(NO_3)_3$ and buffer solutions, and appropriate dilution, concentrations of morin in solutions were 3.03–9.09 ng mL$^{-1}$. Analytical recovery was 100–100.15%. Low values of relative error and relative standard deviation of determination (R.S.D.) indicate very good reproducibility of measurement. The results of the assay are shown in Table 2.

### 3. 2. 3. HPLC Determination

To check the reliability of fluorescence method the direct HPLC determination of morin in serum samples was developed as a modification of reported method.[34] Two calibration curves were established for the determination of morin. One curve was constructed in aqueous phase while the other was obtained from serum. The samples were spiked with various concentrations of morin stock solution (in methanol) to afford of a series of aqueous phase consisting of 1.6, 6.4, 25.0, 50.0, 100.0 and 200.0 μg mL$^{-1}$ of morin.

The calibration curve was obtained by direct injection of working solution into a column. The serum calibration curve was obtained by spiking 200 μL of blank serum containing 100 μL acetate buffer (pH 4.50) by different concentration levels of the standard solution of morin in methanol. The range of morin concentrations thus covered was 1.0 to 200 μg mL$^{-1}$ (6 solutions). The solutions were incubated at 37 °C for 30 min, thoroughly shaken and then 300 μL of the mixture of ether and acetone (90:10 v/v %) was added and the mixture was again shaken on a shaker. The ether layer was evaporated under nitrogen atmosphere and reconstituted with mobile phase and then subjected to HPLC analysis. Validation parameters are given in Table 3.

Recovery was calculated as the ratio between slopes of calibration curves in aqueous phase and in serum. The obtained value of 86% is in acceptable range.

The HPLC method was already applied to determination of morin in mixture of morin and quercetin together with their conjugated metabolites in serum.[34] Spectrofluorimetry enables direct and simple determination of morin without the extraction required for HPLC method. Spectrofluorimetry also provides much lower LOD values than HPLC what undoubtedly is an advantage since nonspecific emission from serum is eliminated by high dilution. HPLC method on the other hand separates morin from protein and other interfering components of serum thus enabling, accurate and precise results. In HPLC method longer incubation time was used leading to metaboli-

**Table 3:** Validation parameters of HPLC determination of morin in human pool serum

| | Serum | Aqueous phase |
|---|---|---|
| Linearity (n = 6) | $Y = (4.29 \pm 0.08)\times10^3\, X + (5.56 \pm 0.08)10^3$ | $Y = (5.0 \pm 0.1)10^3\, X + (10.9 \pm 0.1)10^3$ |
| Y (peak area), X (conc. of morin in μg mL$^{-1}$) | | |
| Coefficient of correlation (r) | 0.9987 | 0.9988 |
| LOD (μg mL$^{-1}$) | 0.056 | 0.055 |
| LOQ (μg mL$^{-1}$) | 0.169 | 0.166 |

te transformations of morin. This provides lower recovery in comparison with the recovery obtain by spectrofluorimetric method.

In comparison with other methods reported,[34,40,41] this method is quick and simple, and has high sensitivity, wide linear range and good stability.

## 4. Conclusions

We obtained that the fluorescence intensity of the aluminium – morin complex, at maximum $\lambda_{em}$= 500 nm, is linear function of morin concentration, in the presence of excess of $Al^{3+}$ ions. On the basis of these results we can propose that the spectrofluorimetric method can be used for quantitative determination of morin in human serum. Reference HPLC method confirmed the results obtained spectrofluorimetrically.

## 5. Acknowledgements

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## 6. References

1. M. G. L. Hertog, D. Kromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti, S. Nedeljkovic, M. Pekkarinen, B. S. Simic, H. Toshima, E. J. M. Feskens, P. C. H. Hollman, M. B. Katan, *Arch. Intern. Med.* **1995**, *155*, 381–386.
2. A. S. Meyer, M. Heinonen, E. N. Frankel, *Food Chem.* **1998**, *61*, 71–75.
3. G. Agullo, L. Gamet-Payrastre, S. Manenti, C. Viala, C. Remesy, H. Chap, B. Payrastre, *Biochem. Pharmacol.* **1997**, *53*, 1649–1657.
4. H. Ashida, I. Fukuda, T. Yamashita, K. Kanazawa, *FEBS Lett.* **2000**, *476*, 213–217.
5. J. An, C. Tzagarakis-Foster, T. C. Scharschmidt, N. Lomri, D. C. Leitman, *J. Biol. Chem.* **2001**, *276*, 17808–17814.
6. D. M. Lepley, J. C. Pelling, *Mol. Carcinog.* **1997**, *19*, 74–82.
7. A. Xagorari, A. Papapetropoulos, A. Mauromatis, M. Economou, T. Fotsis, C. Roussos, *J. Pharmacol. Exp. Ther.* **2001**, *296*, 181–187.
8. N. Bhatia, R. Agarwal, *Prostate* **2001**, *46*, 98–107.
9. R. S. Frey, J. Li, K. W. Singletary, *Biochem. Pharmacol.* **2001**, *61*, 979–989.
10. Y. Nagasaka, K. Nakamura, *Biochem. Pharamcol.* **1998**, *56*, 1151–1155.
11. H. Kobuchi, S. Roy, C. K. Sen., H. G. Nguyen, L. Packer, *Am. J. Physiol.* **1999**, *277*, C403–C411.
12. X. F. Yang, F. Wang, S. S. Hu, *Colloid Surf.* **2006**, *B 52,* 8–13.
13. H. Vogel, M. Gonzalez, F. Fainic, I. Razmilic, J. Rodriguez, J. S. Martin, F. Urbina, *J. Ethnopharmacol.* **2005**, *97*, 97–100.
14. C. Proestos, I.S. Boziaris, G.-J.E. Nychas, M. Komaitis, *Food Chem.* **2006,** *95*, 664–667.
15. Y. C. Fiamegos, C. G. Nanos, J. Vervoort, C. D. Stalikas, *J. Chromatogr. A* **2004,** *1041*, 11–18.
16. T. Wu, Y.Q. Guan, J. N. Ye, *Food Chem.* **2007**, *100*, 1573–1579.
17. X. Q. Xu, H. Z. Ye, W. Wang, L. S. Yu, G.N. Chen, *Talanta* **2006**, *68*, 759–764.
18. X. Q. Xu, L. S. Yu, G. N. Chen, *J. Pharm. Biomed. Anal.* **2006**, *41*, 493–499.
19. M. Polasek, I. Petriska, M. Pospisilova, L. Jahodar, *Talanta* **2006***, 69,* 192–196.
20. L. Ch. Chang, S. W. Sun, *J. Pharm. Biomed. Anal.* **2006***, 40*, 62–67.
21. J. J. Liu, S. P. Li, Y. T. Wang, *J. Chromatogr. A* **2006***, 1103* (2) , 344–349.
22. A. Doménech-Carbo, M.T. Doménech-Carbo, M.C. Saurí-Peris, *Talanta* **2005***, 66,* 769–782.
23. S. Ignatov, D. Shishniashvili, B. Ge, F.W. Scheller, F. Lisdat, *Biosens.Bioelectron.* **2002***, 17*, 191–199.
24. A. J.Wilko³azka, T. Ruzgas, L. Gorton, *Enzyme Microb. Technol.* **2004***, 35*, 238–241.
25. U. Justesen, P. Knuthsen, T. Leth, J. *Chromatogr. A,* **1998***, 79*, 101–110.
26. C. S. Lau, D. J. Carrier, R. R. Beitle, D. I. Bransby, L. R. Howard, J. O. Lay Jr., R. Liyanage, E. C. Clausen, *Bioresour. Technol.* **2007***, 98*, 429–435.
27. H. F. Wang, K. H. Well, *Food Res. Int.* **2001**, *34,* 223–227.
28. F. Fang, J. M. Li, Q. H. Pan, W. D. Huang, Food Chem. **2007,** *101*, 428–433.
29. B. Liu, D. Anderson, D. R. Ferry, L. W. Seymour, P. G. de Takats, D. J. Kerr, *J. Chromatogr. B* **1995,** *666*, 149–155.
30. A. C. Gutierrez, M. H. Gehlen, *Spectrochimica Acta Part A* **2002,***58,* 83–89.
31. R. Ghavami, A. Najafi, B. Hemmateenejad, *Spectrochimica Acta Part A* **2008***, 70* 824–834.
32. T. Sawada, T. Shibamoto, H. Kamada, *Bull. Chem. Soc. Jpn.* **1978**, *51(6)*, 1736–1738.
33. P. C. H. Hollman, J. M. P. van Trijp, M. N. C. P. Buysman, *Anal. Chem.* **1996,** *68*, 3511–3515.
34. S. L. Hsiu, C. W. Tsao, Y. C. Tsai, H. J. Ho, P. D. L. Chao, *Biol. Pharm. Bull.* **2001.** *24(8),* 967–969.
35. D. D. Perrin, B. Dempsey: Buffers for pH and Metal Ion Control; Chapman and Hall, London, **1974,** pp 77–94.
36. J. Yoe, A. Jones, *Ind. Eng. Chem. Anal. Ed.* **1944,** *16,* 111–115.
37. H. Irving, T. Pierce, *J. Chem. Soc.* **1959,** 2565–2574.
38. J. N. Miller, J. C. Miller: In Statistics and Chemometrics for Analytical Chemistry, 5th edn., Pearson Education Ltd: London, **2005**, p. 121.
39. Validation of analytical procedures: Methodology, ICH Guideline Q2B, **1997,** Federal Register 62, No 96, pp. 27463–27467.

40. F. Fang, J. M. Li, Q. H. Pan, W. D. Huang, *Food Chemistry* **2007,** *101*, 428–433.

41. P. Xiao, Q. Zhou, F. Xiao, F. Zhao, B. Zeng, *Int. J. Electrochem. Sci.* **2006,** *1*, 228–237.

## Povzetek

Morin je flavonoidni antioksidant. V mešanici etanol – voda (70 ut. % etanola) reagira z $Al^{3+}$, pri čemer nastane v pH območju med 3 in 6 kompleks $Al(Morin)_2$. Logaritem pogojne konstante stabilnosti ($\beta_2$) tega kompleksa je pri 298 K in pH 16.96 ± 0.02. Kompleks kaže pri vzbujevalni valovni dolžini 410 nm fluorescenčni emisijski maksimum pri 500 nm. Intenziteta je odvisna od pH in je največja pri pH 4.40. Ker nastanek kompleksa poveča fluorescenco morina, je bila ta lastnost uporabljena za določanje morina v človeškem serumu. Ugotovljena je bila linearna odvisnost intenzitete emitirane svetlobe od koncentracije morina v območju med 1.5 ter 30.5 ng $mL^{-1}$. Relativna standardna napaka meritev je pri bila 1.4 %, meja zaznave 0.02 ng $mL^{-1}$ ter meja kvantifikacije 1.0 ng $mL^{-1}$. Koncentracije morina v seruma so bile določene tudi z referenčno HPLC metodo z uporabo C-18 Hypersil Gold AQ kolone in mobilno fazo acetonitril –0.1 % fosforna kislina (30:70 % v/v) pri pretoku 1.0 mL $min^{-1}$ in UV detekcijo pri 250 nm. Sprejemljive relativne standardne napake (manj kot 5 %) dobljene na osnovi primerjave rezultatov z referenčno HPLC metodo potrjujejo zanesljivost fluorescenčne metode.