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RESEARCH PAPER

Potentiometric and Chromatographic Study of Cu(II) and Al(III) Complexes of Quercetin

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ABSTRACT

In this study, the stability constants of the copper(II) and aluminium(III) complexes of quercetin were determined potentiometrically by using Calvin-Bjerrum and Irwing Rossotti methods. The protonation constants of quercetin were found: $\log K_1$ = 11.15±0.118, $\log K_2$ = 10.42±0.144, $\log K_3$ = 9.44±0.162, $\log K_4$ = 8.28±0.151. For copper complex formation constant was found by Irwing-Rossotti method: $\log K_1$ = 19.92±0.367 and for aluminium complex complex: $\log K_1$ = 23.02±0.459. From the results, the components of quercetin / metal complexes are given as 1/1 both copper(II) and aluminium(III).

A reversed phase high pressure liquid chromatographic method was developed for the metal complexes which were prepared according to potentiometric results with the ratio quercetin / metal =1/1. Mobile phase was 0.01 M $HClO_4$ / 8.33 x 10^{-5} M quercetin in Methanol (40/60), column was XTerra RP18, 5 µm, 4.6 x 150 mm , detector was Diode Array Detector at λ = 373 ve 421 nm (band width 4nm).

Keywords: Flavonoid-metal complexes, quercetin complexes, biologically active ligands, antioxidants, flavonoids.

Introduction

Flavonoids are a group of polyphenolic natural compounds widely distributed in the plant kingdom. They are present in fruits, vegetables and foods of plant origin. Recent years there has been much interest in flavonoids because of their beneficial effects on human health: e.g. antioxidant activity, free radical scavenging capacity, anti-inflammatory, antimicrobial, antiviral, hypolipidemic, antimutagenic effects and anticarcinogenic potential (1). These activities are mainly attributed to their powerful antioxidant activity and modulation of enzymatic activities (2).

Quercetin (3,5,7,3',4'-pentahydroxyflavone, Figure 1), part of a subclass of flavonoids called flavonols, has received considerable attention because of its overwhelming presence in foods. Flavonols consist of two aromatic rings (A and B rings) linked by a 3-carbon chain that forms an oxygenated heterocyclic ring (C ring) and hydroxyl group substituted in 3 position. A number of functional groups mainly hydroxyl can be attached to ring structures of flavonols. Among polyphenols, quercetin is the most important and naturally occurring cancer-preventing agent (3). The cancer preventive and therapeutic effects of quercetin have been demonstrated through *in vitro* as well as *in vivo* experimental findings (4, 5).

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Metal ions have important roles in biological process in cells as electrolytes, structural and functional activities with proteins, enzymes. In recent years, metal–organic complexes have drawn much attention because many organic molecules can coordinate with various metal ions to form metal complexes with diverse geometries, providing infinite possibilities for coordination numbers and applications (6, 7). The widespread success of cisplatin in the clinical treatment of various types of tumor has placed coordination chemistry of metal-based drugs in the frontline in the fight against cancer. The clinical success of cisplatin has proved to be limited due to significant side effects. Therefore, medicinal inorganic chemistry has focused on developing new chemotherapeutic metal complexes with improved properties. Flavonoids are effective metal ion chelators. The interaction of flavonoids with metal ions may change the antioxidant properties and enhance the pharmacological properties of the flavonoids (8, 9). Current studies about metal-flavonoid complexes are mainly focused on their anticancer, antioxidant or prooxidant, antimicrobial, antiproliferative activities and their interaction with DNA (10, 11).

Molecular structure of Fe(II), Fe(III), Cu(II), Cd(II), Zn(II), Pb(II), Co(II), Sn(II), Al(III), complexes of quercetin was intensively studied using spectroscopic methods (UV–Vis, IR, Raman, NMR, ESI-MS, MALDI-TOF MS) (12-15). Chromatographic methods for the separation and determination of metal complexes are mainly used in trace metal analysis. However, in spite of practical achievements, the problem on a quantitative basis still remains. In chromatographic system dissociation, hydrolysis, ion or ligand exchange can make the separation difficult. The problem is concerned with the stability of the complexes (16, 17). Therefore, in this study, the stability constants of the copper (II) and aluminium(III) complexes of quercetin were determined potentiometrically by using Calvin-Bjerrum and Irwing Rossotti methods (18, 19). An analytical procedure for the determination of Cu(II) and Al(III) complexes of quercetin by reversed phase HPLC is presented.

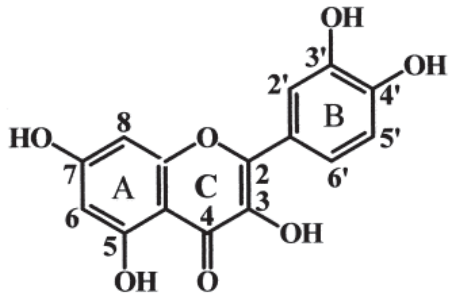

Figure 1. Chemical structure of quercetin.

Materials and Methods

Materials: $HClO_4$, 0.0100 M NaOH solution (titrisol), $NaClO_4$, 1,4-Dioxane, $Al(NO_3)_3.9H_2O$, $CuSO_4.5H_2O$, Methanol, pH 4.00 and pH 7.00 buffer solutions, were purchased from Merck. Quercetin was purchased from Carl Roth. Quercetin solution was prepared in dioxane-water system (1:1, v/v).

Potentiometric Method: The protonation constants of quercetin and the stability constants of Cu(II) and Al(III) complexes of quercetin, were determined potentiometrically using the Calvin-Bjerrum method, and calculations were performed according to the Irving-Rossotti equations in Microsoft Excel.

Potentiometric titrations were carried out using a Radiometer TitraLab 80 automatic titrator equipped with a Radiometer PHC3001-8 combination pH electrode. The electrode was calibrated daily using standard buffer solutions. The titrations were performed in a dioxane-water system (1:1, v/v) at constant ionic strength (0.1 M $NaClO_4$ solution I=0.11) at 25°C and a total volume of 25 mL was used for each titration. The following solutions were prepared and titrated potentiometrically against standard 0.0100 M NaOH solution:

a)2.5 mL of 0.01 M $HClO_4$ + 2.5 mL of 0.1 M $NaClO_4$

b)5 mL of 12.10^{-3} M Quercetin + 2.5 mL of 0.01 M $HClO_4$ + 2.5 mL of 0.1 M $NaClO_4$ (for the determination of the protonation constants of quercetin)

c)2.5 mL of 12.10^{-3} M Al(III) + 5 mL of 12.10^{-3} M Quercetin + 2.5 mL of 0.01 M $HClO_4$ + 2.5 mL of 0.1 M $NaClO_4$ (for the determination of the stability constant of quercetin-Cu(II) complex)

d) 2.5 mL of 12.10^{-3} M Cu(II) + 5 mL of 12.10^{-3} M Quercetin + 2.5 mL of 0.01 M $HClO_4$ + 2.5 mL of 0.1 M $NaClO_4$ (for the determination of the stability constant of quercetin-Al(III) complex)

To determine the protonation constants, the solutions of a and b were titrated potentiometrically using 0.01 M NaOH. Potentiometric titration curves 1 and 2 (Figure 2) of each system were used to calculate the average values $\overline{n}_A$. The equation used for the calculation is

$$\overline{n}_A = y + [(V_1 - V_2)(N + E^o)] / [(V^o + V1)\, T^o_L]$$

where V^o is the initial volume (25.0 mL); N is the molarity of NaOH (0.0100 M); T^o_L is the ligand concentration (0.0024 M); E^o is the initial concentration of the $HClO_4$ (0.0122 M);

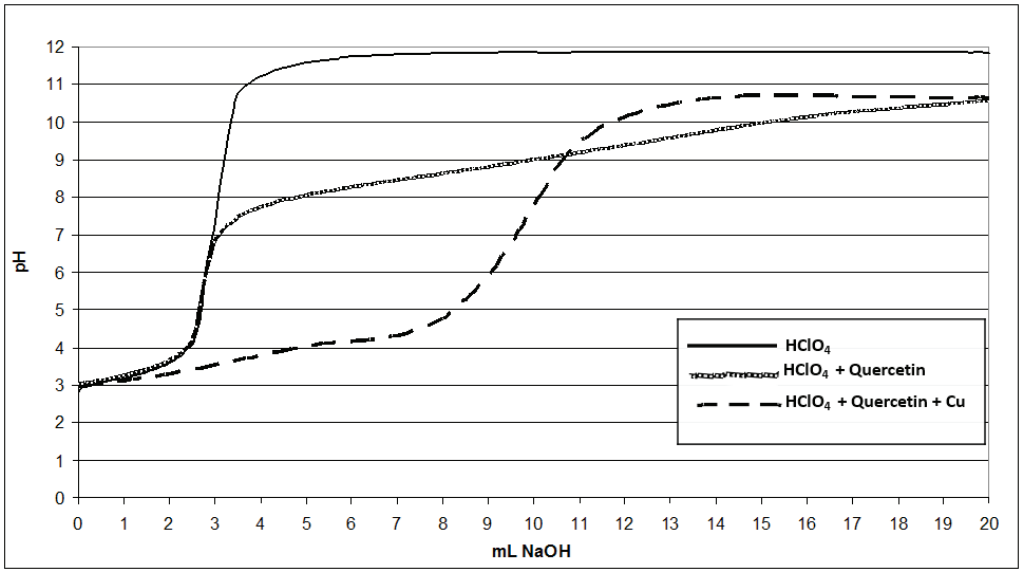

Figure 2. Potentiometric titration curves for the quercetin-Cu(II) system.

and y denotes the number of dissociable protons initially present on the ligand (y=4); (V_1 - V_2) is the measure of the displacement of the ligand curve relative to the acid curve where V_1 and V_2 are the volumes of NaOH added to reach the same pH reading in both titrations.

The protonation constants were determined from the graph of $\overline{n}_A$ vs pH (Figure 3), the pH values at $\overline{n}_A$= 0.5, $\overline{n}_A$= 1.5, $\overline{n}_A$= 2.5, $\overline{n}_A$= 3.5 designate the logK_1, log K_2, log K_3, log K_4 respectively (values are reported in Table 1).

To determine the stability constants of the complexes, the solutions of c and d were titrated potentiometrically using 0.0100 M NaOH. (curve 3 in Figure 2 and Figure 5). $\overline{n}_L$ values were calculated using the $\overline{n}_A$ values and the equation given below:

$$\overline{n}_L = [(V_3-V_2)(N+E^o+T^o_L(y-\overline{n}_A)] / (V^o+V_2).$$

where V^o is the initial volume (25.0 mL); N is the molarity of NaOH (0.0100 M); T^o_L is the ligand concentration (0.0024 M); E^o is the initial concentration of the $HClO_4$ (0.0122 M);

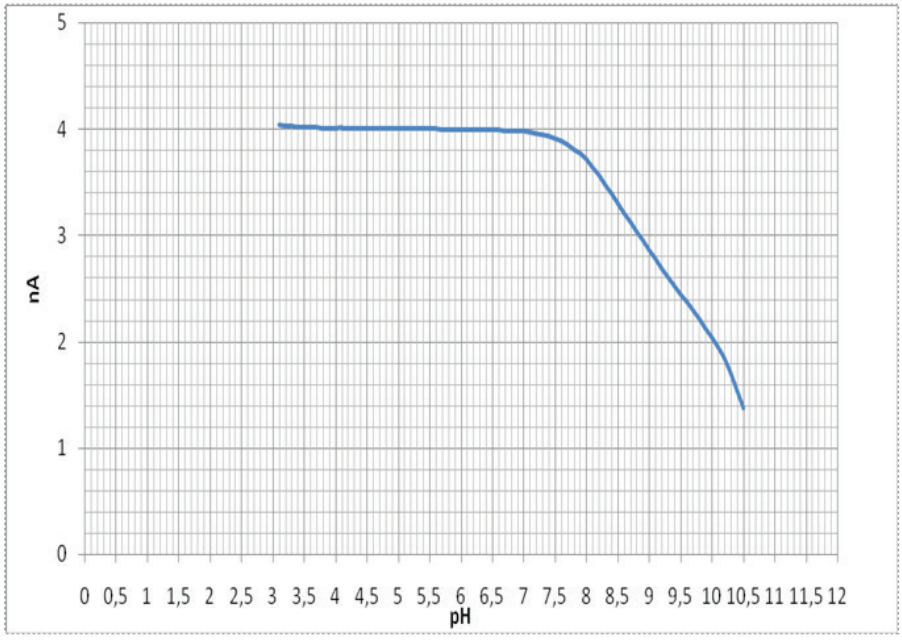

Figure 3. $\overline{n}_A$ vs pH plots of quercetin.

Table 1. Protonation constants of quercetin and stability constants of the Cu(II) and Al(III) complexes of quercetin.

	$logK_1$	$logK_2$	$logK_3$	$logK_4$
Protonation constants of quercetin	11.15±0.118	10.42±0.144	9.44±0.162	8.28±0.151
Stability constant of Quercetin-Cu(II) complex	19.92±0.367	-	-	-
Stability constant of Quercetin-Al(III) complex	23.02±0.459	-	-	-

T^b_M is the metal concentration (0.0012 M); y denotes the number of dissociable protons initially present on the ligand (y=4); (V_3 - V_2) is the measure of the displacement of the metal curve relative to the ligand curve where V_2 and V_3 are the volumes of alkali added to reach the same pH reading. pL values were calculated using the $\bar{n}_L$ values and the equation given below:

$pL = \log(1+\beta_1[H^+] + \beta_2[H^+]^2 + \beta_3[H^+]^3 + \beta_4[H^+]^4) / (T^b_L - \bar{n}_L T^b_M)$

$\beta_1 = K_1 = 1{,}41.10^{11}$

$\beta_2 = K_1.K_2 = 1.41.10^{11} . 2.66.10^{10} = 3.75.10^2$

$\beta_3 = K_1.K_2.K_3 = 1.41.10^{11} . 2.66.10^{10} .2.73.10^9 = 1.03. 10^{31}$

$\beta_4 = K_1.K_2.K_3.K_4 = 1.41.10^{11} . 2.66.10^{10} .2.73.10^9 . 1.9. 10^8 = 1.95. 10^{39}$

The stability constants were determined from the $\bar{n}$ vs pL curve, where the pL values at $\bar{n}_L = 0.5$ designate the log K_1. Figure 4 and Figure 6 show the formation curves for Cu(II) and Al(III) complexes of quercetin respectively. And their stability constant values are reported in Table 1.

HPLC method: The stoichiometry of Cu(II) and Al(III) complexes of quercetin was determined by potentiometric method. Complexes were prepared by mixing stoichiometric amounts of quercetin and $Al(NO_3)_3.9H_2O$, quercetin and $CuSO_4.5H_2O$ in methanol, to reach the molar ratio M:L (1:1). A concentration of 5 x 10^{-3} M quercetin was used.

Experiments were carried out on a chromatographic system Agilent 1100 equipped with binary gradient pump, degasser, autosampler, column thermostat and diode array a detector (Agilent Technologies). Data acquisition and processing were controlled by Chemstation software (Agilent Technologies). Chromatographic column XTerra RP18, 5 µm, 4.6 x 250 mm. (Waters) was used for the chromatographic separation.

An HPLC analysis was performed by isocratic elution with a flow rate of 0.7 mL/min at 30°C. The mobile phase consisted of 0.01 M $HClO_4$ / 8.33 x 10^{-5} M Quercetin solution in

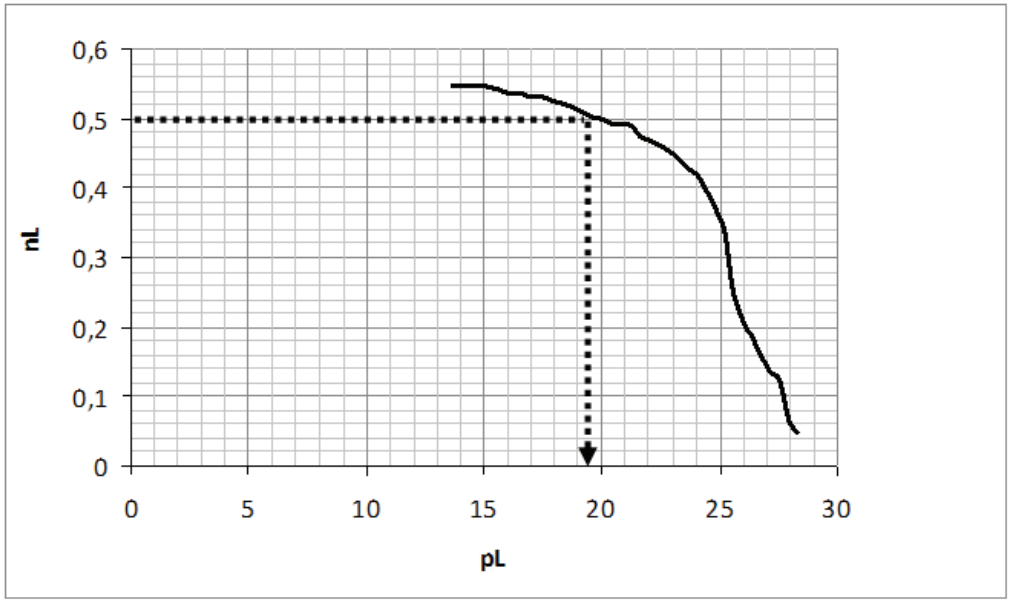

Figure 4. nL vs pL plots of quercetin-Cu(II) complex.

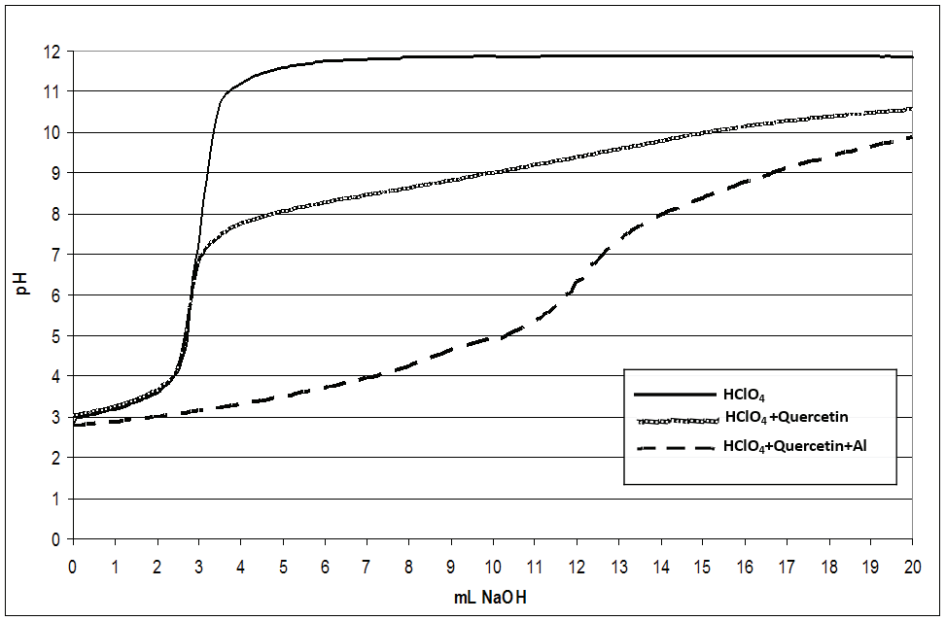

Figure 5. Potentiometric titration curves for the quercetin-Al(III) system.

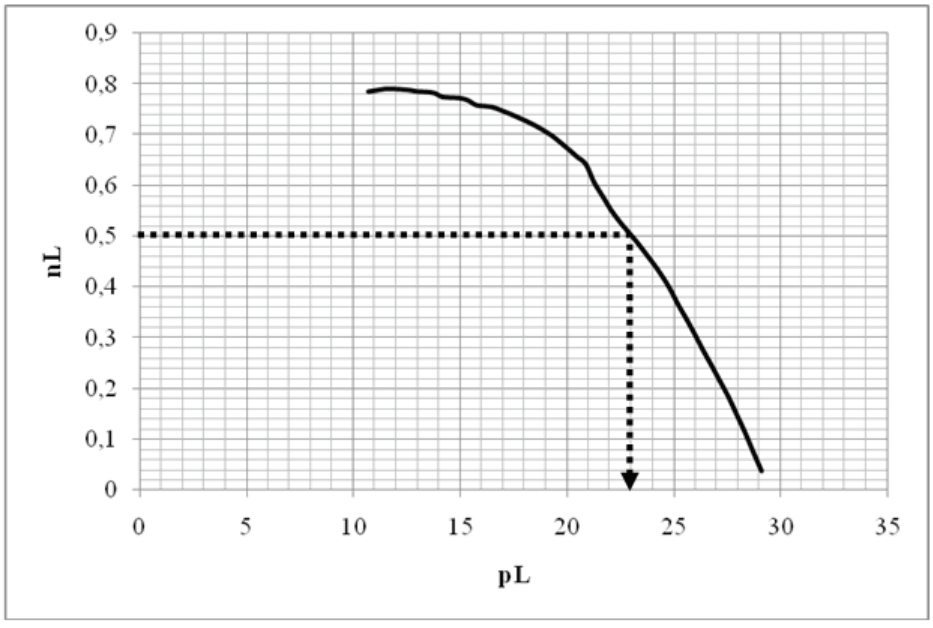

Figure 6. nL vs pL plots of quercetin-Al(III) complex.

methanol (40/60). DAD detector was set at λ = 373± 4nm, 421± 4nm. And the injection volume was 50 µL.

Results and Discussion

The stability constants of the Cu(II) and Al(III) complexes of quercetin were determined potentiometrically by using Calvin-Bjerrum and Irwing Rossotti methods (Table 1). From the results, the components of quercetin/metal complexes are given as 1/1 for both Cu(II) and Al(III).

The work of H. Lian *et al* indicated that the Morin-Aluminum complex was satisfactorily separated on a Spherisorb 18 ODS 2, 5 mm, 150x4.6 mm column using mobile phase as methanol–water (30:70) adjusted to pH 1.0 with perchloric acid [16]. Perchloric acid was chosen due to low complexation power to Al. A higher acidity of the mobile

phase could suppress the dissociation of the complex on to column. Based on these information XTerra RP18, 5 µm, 4.6 x 250 mm. (Waters) column that enables working at wide pH range (pH=2 – pH=10) was chosen for separation. On this column, separation of quercetin-Al(III) complex was best achieved with the mobile phase of 0.01 M $HClO_4$ (pH 2.05)/ Methanol (40 / 60, v/v). Figure 7 shows the chromatogram of quercetin and Figure 7a shows the DAD spectrum of the quercetin peak. Figure 8 shows the quercetin-Al(III) complex. Comparing the spectrums at Figure 7a and Figure 8a corresponding to these two peaks, the maximum absorption wavelengths are 373 nm and 421 nm, respectively. Addition of Al(III) to quercetin caused a bathochromic shift which proves formation of the complex.

Whereas for less stable Cu(II)-quercetin complex, no chromatographic peak was obtained. Attempts to determine the Cu(II)-quercetin complex, changing the concentration of the $HClO_4$, organic composition of the mobile phase, using buffer solutions with different pH, counter ions in aqueous part of mobile phase were unsuccessful, no peaks determined.

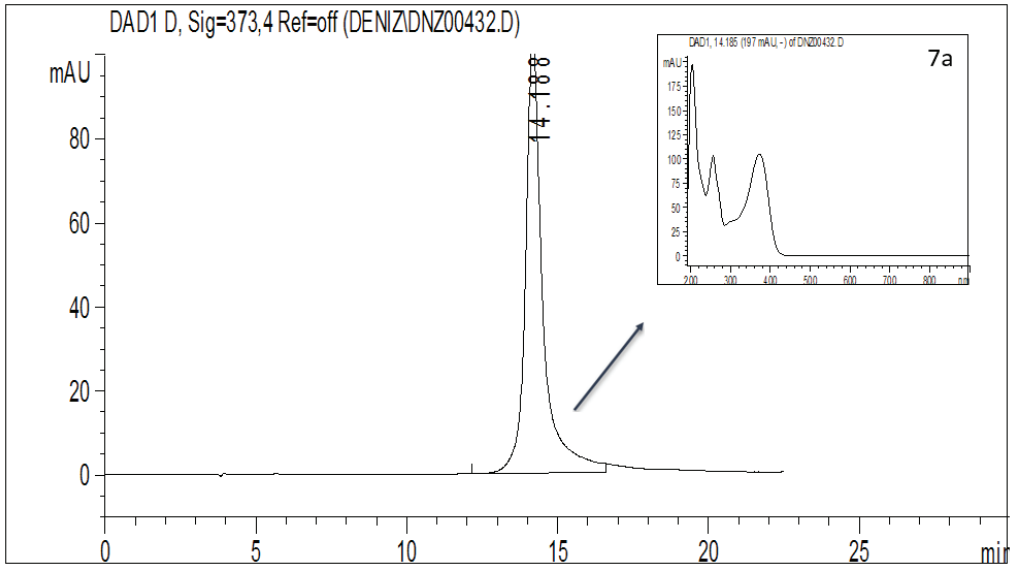

Figure 7. Chromatogram of quercetin. Chromatographic conditions are: Mobile phase 0.01 M $HClO_4$ / methanol (40/60). Flow rate = 0.7 mL/ min at 30 °C. XTerra RP18, 5 µm, 4.6 x 250 mm. column.

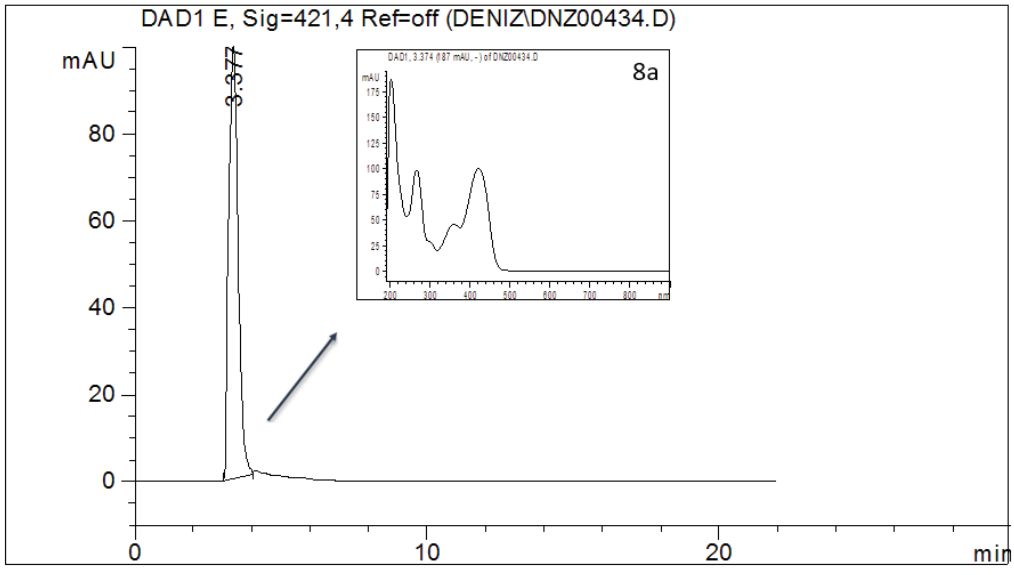

Figure 8. Chromatogram of quercetin-Al(III) complex. Chromatographic conditions are: Mobile phase 0.01 M $HClO_4$ / methanol (40/60). Flow rate = 0.7 mL/min at 30 °C. XTerra RP18, 5 µm, 4.6 x 250 mm. column.

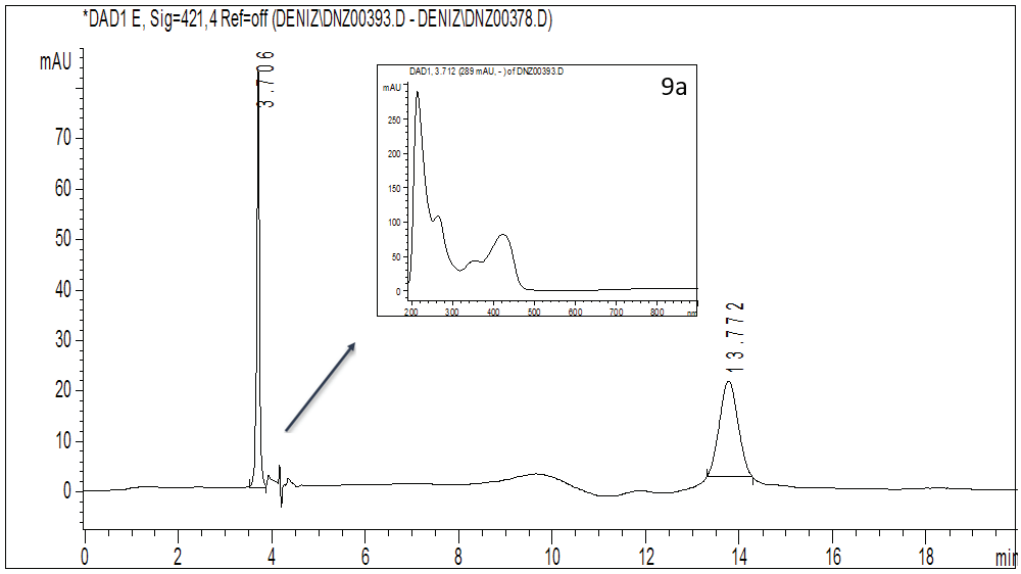

Figure 9. Chromatogram of quercetin-Cu(II) complex.T_R = 3.706. Chromatographic conditions are: Mobile phase 0.01 M $HClO_4$ / 8.33 x 10^{-5} M Quercetin solution in methanol (40/60). Flow rate = 0.7 mL/min at 30 °C. XTerra RP18, 5 µm, 4.6 x 250 mm. column.

Some additional work was carried out with the addition of free ligand to the mobile phase based on the study of S. Dilli *et al* (20). Because of the insolubility in water, quercetin was added in methanol phase. The mobile phase consisted of 0.01 M $HClO_4$ / 8.33 x 10^{-5} M Quercetin solution in methanol (40/60). To investigate the effect of quercetin in the mobile phase on separation, various concentrations were tested. A concentration of 8.33 x 10^{-5} M was chosen according to sharpest peak shape and peak symmetry. Figure 9 shows besides the unreacted quercetin peak (T_R=13.772) the baseline substracted chromatogram of quercetin-Cu(II) complex with the retention time 3.706. Figure 7a and Figure 9a corresponding to quercetin and quercetin-Cu(II) complex, the maximum absorption wavelengths are 373 nm and 421 nm, respectively. Addition of Cu(II) to quercetin caused a bathochromic shift which proves formation of the complex.

As a conclusion, potentiometric titrations has showed the strong complexation with a 1:1 stoichiometry (M:L) for both Cu(II) and Al(III). The stability constants of the Cu(II) and Al(III) complexes of quercetin are quite high with the values log K_1=19.92±0.367 and log K_2= 23.02±0.459. Stability of the aluminium complex is higher than copper complex. A Reversed phase HPLC separation method was developed for identifying these complexes. Based on potentiometric results, metal complexes were prepared in methanol with a 1:1 stoichiometry. The main limitation was dissociation of the less stable Cu(II)-quercetin complex on chromatographic column. This limitation was overcome by addition of free ligand to the mobile phase.

Kuersetinin Cu(II) ve Al(III) Komplekslerinin Potansiyometrik ve Kromatografik İncelenmesi

ÖZ

Bu çalışmada, Kuersetinin bakır(II) ve alüminyum(III) ile oluşturduğu komplekslerin kararlılık sabitleri Calvin-Bjerrum ve Irwing-Rossotti yöntemleri kullanılarak potansiyometrik yoldan tayin edildi. Kuersetin dissosiyasyon sabitleri potansiyometrik yöntemle logK_1 = 11.15±0.118, logK_2 = 10.42±0.144, logK_3 = 9.44±0.162, logK_4 = 8.28±0.151 olarak bulundu. Yine potansiyometrik olarak Irwing-Rossotti oluşum sabitleri ise bakır(II) için logK_1 = 19.92±0.367, alüminyum(III) için logK_1 = 23.02±0.459 olarak bulundu. Bakır(II) ve alüminyum(III) komplekslerinin bileşimleri ligand/metal = 1/1 olarak bulundu.

Potansiyometrik sonuçlara dayanarak kuersetin/metal = 1/1 oranıyla hazırlanan kompleksler için ters fazlı yüksek basınçlı sıvı kromatografisi ile tayin yöntemi gerçekleştirildi. Çalışmada hareketli faz olarak 0.01 M $HClO_4$ / 8.33 x 10^{-5} M Kuersetin çözeltisi (Metanolde) (40/60), XTerra RP18, 5 µm, 4.6 x 150 mm özellikte kolon, λ = 373 ve 421 nm (bant genişliği 4nm) kullanıldı.

Anahtar kelimeler: Flavonoid-metal kompleksleri**,** kuersetin kompleksleri, biyolojik aktif ligandlar, antioksidanlar, flavonoidler.

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