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INDIRECT ATOMIC ABSORPTION SPECTRO-METRIC METHOD FOR THE DETERMINATION OF RUTIN IN ITS PURE AND PHARMACEUTICAL DOSAGE FORMS.

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ABSTRACT

Indirect method for the determination of Rutin (quercetin-3-rutinoside) was proposed. The method is based on precipitating ion association complexes of rutin with $[Co(NH_3)_5Cl]^{2+}$, $[Cu(NH_3)_4]^{2+}$ *and* $[Cd(NH_3)_4]^{2+}$ *and the excess, unreacted Co, Cu and Cd complex ions were determined using Atomic Absorption Spectrometry. The method was successfully used for determining Rutin in its pure and pharmaceutical dosage forms. The drug was determined in concentration range 0f 5.6-56.8 mg with average relative standard deviation of 0.91-1.16% and recovery percent of 100.21-101.10%. These results indicated high precision and accuracy for the proposed method and its suitability for routine quality control analysis of this drug and similar drug compounds.*

INTRODUCTION

Rutin (quercetin-3-rutinoside) [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-3-yl rutinoside] [153-18-4] has a direct constructor action on the capillary bed and decreases the permeability and fragility of the vessels [1]. It protects against capillary injury [2-3] and produces a lowering of blood pressure [4]. Antimicrobial activity of Rutin was also reported [5]. Therapeutic uses of Rutin is concentrated in the treatments of capillary fragility, retinal hemorrhage, some hereditary hemorrhagic disorders such as hemophilia, bleeding gums, migraine headaches and toxemia in pregnancy [6]. Rutin as all biflavonoids stimulates the production of blood platelets and is recommended in treatment of thrombopenia [5].

Several techniques have been used for authentic Rutin determination. These techniques included colorimetric determination based on forming

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colored derivative by chelation with $AlCl_3$ and measuring the absorption of the solution at 416 nm ; Be^{2+} forms with Rutin yellow complex measured at 465 nm; $TiOSO_4$ gives yellow orange complex and uranyl acetate gives orange complex and Cu^{2+}, Ga^{3+} and Sb^{3+} were also reported to give colored complexes which can be measured colorimetrically [5]. Electrophilic substitutions with aminobenzoic acid followed by measuring the colored product at 420 and 410 nm, respectively were also reported [7-8].

Rutin has been determined in pharmaceutical preparations by measuring its absorption at 256 and 360 nm [9-10] and by measuring the first UV derivative spectrophotometry [11]. A rapid PMR procedure was also mentioned [5]. Other methods based on fluorimetric, polarographic, densitometric, gravimetric and several types of chromatographic procedures and electrophoresis were reported for Rutin determination [5].

Atomic Absorption Spectrometry (AAS) occurs in the fore front of the most sensitive and widely used analytical techniques. The reasons might be attributed to the very low detection limit, simplicity, reproducibility and low running costs which are advantages compared to a large number of other analytical tools.

The aim of this investigation is to develop a new method for Rutin determination using Atomic Absorption Spectrometry. This method is based on precipitating ion association complexes between Rutin and each of $[Co(NH_3)_5Cl]^{2+}$, $[Cu(NH_3)_4]^{2+}$ and $[Cd(NH_3)_4]^{2+}$. The excess, non-reacted, Co, Cu and Cd ions present as soluble inorganic complex ions in the supernatant solutions were determined using AAS. This method will be compared with the pharmacopoeial accredited and newly published methods.

EXPERIMENTAL

Apparatus

The pH values were measured using Orion Research digital pH meter, Model 601A with accuracy ± 0.05 pH units.

AAS measurements were carried out using Shinadzu Atomic Absorption Spectrometer, type AA-625.

Conductometric measurements were carried out using YSI model 32 M conductivity meter with cell constant K=1, (TOA Electronic Ltd., Japan).

Materials

Double distilled water from glass wares was used. All reagents were of analytical reagent grades. Cobalt chloride, copper sulfate and cadmium nitrate were supplied by Aldrich.

Pharmaceutical Preparations

Authentic Rutin samples with 99.5% purity were provided by Misr Company for Pharmaceutical Industries, Egypt.

Ruta-C-60 Tablets, (60 mg Rutin / Tablet), Kahira Pharmaceuticals and Chemical Industries Co., Cairo, Egypt, purchased from local Market.

Rutin-C, (50 mg Rutin / Tablet), Pharco Pharmaceuticals Co., Alexandria, Egypt, purchased from local Market.

Reagents

$[Co(NH_3)_5Cl](ClO_4)_2$, $[Cu(NH_3)_4](ClO_4)_2$ and $[Cd(NH_3)_4](ClO_4)_2$ were prepared using the standard procedure [12]. To adjust the ionic strength, 2.5, 3.75 and 6.25 ml (1M NaCl stock solution) were diluted to 25 ml and adjusted to the required pH were used as filling solutions in determinations of Co, Cu and Cd, respectively.

Standard Solutions

Standard solutions of Co(II), Cu(II) and Cd(II) were prepared by weighing 1.0 gram of pure cobalt chloride, copper sulfate or cadmium nitrate and transferring it into 1000 ml measuring flask. 50 ml of concentrated nitric acid was added. The solution was well shaken to dissolve the metal salt and completed to the mark with distilled water. The resulting solutions were stored in plastic bottles which were presoaked in dilute nitric acid. Such solutions are stable for approximately one year.

Analytical Procedures

Measurement Parameters

Co(II), Cu(II) and Cd(II) were measured by AAS in the absorption mode using Air-C_2H_2 at 240.7, 324.7 and 228.8 nm and slit widths of 1.9, 1.9 and 1.9 Å, respectively. The relative noise was 1.0 with absorption sensitivities of 0.16, 0.13 and 0.025 µg/ml, respectively. The lamp currents were 10, 10 and 6 mA with burner heights of 6, 4 and 3 cm and gas flows of 6, 2.3 and 1.3 (air/acetylene) for Co, Cu and Cd, respectively. The integration time was about 3 seconds.

Calibration Graphs

Calibration graphs were constructed using the standard cobalt, copper and cadmium solutions in 1 M nitric acid previously mentioned. Solutions containing 1, 2, 3...up to 20 μg/ml metal ion concentration were measured. Graphs of absorption versus concentration in ppm were plotted. Each line was measured three times to check the reproducibility.

Stoichiometric Ratio

Stoichiometric composition of the associate complex was identified by conductometric titration [13]. 50 ml (1.6×10^{-4} M) Rutin dissolved in methyl alcohol with was titrated with 10^{-3} M from each of $[Co(NH_3)_5Cl](ClO_4)_2$, $[Cu(NH_3)_4](ClO_4)_2$ or $[Cd(NH_3)_4](ClO_4)_2$. A 1:2 associate complex (ion:Rutin) was obtained in all cases (Fig. 1).

Preparation of Ion Associate Complexes

Ion associates were prepared by mixing 0.001 mole of each of $[Co(NH_3)_5Cl](ClO_4)_2$, $[Cu(NH_3)_4](ClO_4)_2$ or $[Cd(NH_3)_4](ClO_4)_2$ with 0.002 moles of Rutin in methyl alcohol The precipitated complexes were filtered, washed with distilled water and dried at room temperature. Confirmation for composition was done by C, H, N and metal content [14] elemental analysis (Table 1).

Effects of pH and Ionic Strength

Effects of pH and ionic strength on the solubility of prepared associate complexes were examined by measuring their solubility in terms of its free metal ion concentration by AAS at different pH and ionic strength values (Table 1). The lowest solubility was recorded at values of 0.2-0.5 and 3-4 for ionic strength and pH, respectively [15].

Determination Procedures

Authentic sample Assay (General Procedure)

Successive aliquots of 0.5 to 10 ml of 10^{-2} M methanolic Rutin solution were transferred into 25 ml measuring flasks. To each flask, 10 ml (10^{-2} M) standard aqueous solution of each of $[Co(NH_3)_5Cl](ClO_4)_2$, $[Cu(NH_3)_4](ClO_4)_2$ or $[Cd(NH_3)_4](ClO_4)_2$ was added. The flasks were

completed to the mark with solutions of the optimum pH and ionic strength. The resulting solutions were shaken very well and left to stand for 15 minutes. The precipitates were filtered through Whatman P/S filter paper (12.5 cm). Excess metal ions in the filtrate were determined by AAS using the previously mentioned measurement parameters. The metal ions consumed in the ion associate complex formation were calculated by subtraction. This value is equivalent to half the Rutin concentration in the sample. Thus Rutin was indirectly determined.

Pharmaceutical preparations Assay

To determine Rutin in pharmaceutical preparations, 12 tablets of Ruta-C-60 or 20 tablets of Rutin-C were carefully grind to fine powder. 0.02 to 1.0 gm powder aliquots of Ruta-C-60 or Rutin-C, were dissolved in methanol and quantitatively transferred to 25 ml measuring flasks. Then the analysis was performed as in the general procedure.

RESULTS AND DISCUSSION

Elemental analysis and metal content determination given in Table 1 showed that Rutin forms with the selected complex ions 1:2 (reagent:drug) associate complexes. Comparing the solubility of different associates at the same pH and ionic strength showed that, $[Co(NH_3)_5Cl]^{2+}$ forms with Rutin the most stable associate complex (pK_{sp} = 14.42) whereas $[Cd(NH_3)_4]^{2+}$ forms intermediate stable (pK_{sp} = 13.89) and $[Cu(NH_3)_4]^{2+}$ forms the least stable complex (pK_{sp} = 13.63).

Table 2. shows the stability optimum pH and ionic strengths for the prepared associates in terms of its solubility and solubility product values. The results of solubility measurements revealed that pH values between 3 and 4 are the optimum for the stability of the three associates. $[Co(NH_3)_5Cl]^{2+}$ forms stable associates with Rutin at 0.2 ionic strength whereas $[Cu(NH_3)_4]^{2+}$ and $[Cd(NH_3)_4]^{2+}$ showed highest stability at 0.3 and 0.5 ionic strengths, respectively [15].

Conductometric Titration

Stoichiometric composition of associate complexes were further confirmed by conductometric titration [17]. 1:2 (reagent:drug) were obtained for all treated metal ions (Fig. 1).

Reaction Mechanism

The mechanism of formation of the associate complexes from Rutin and the corresponding metal complex ion is shown in Fig. 2. Rutin is hydrolyzed to Quercetin which is monovalent negatively charged ion. In presence of divalent metal ion complex, associate complexes are precipitated with the formula given in Table 1.

Calibration Graph and Concentration Range

AAS measurements for the three series of standard solutions of Co, Cu and Cd were measured. Calibration graphs for the three elements showed straight lines over dynamic concentration ranges of 3-12, 2-8 and 0.5-2 μg/ml for Co, Cu and Cd, respectively. The corresponding regression equations gave correlation coefficients in the range of 0.99 - 1.05.

Analytical Determination of Rutin in Authentic Samples

Rutin was accurately determined using the proposed method in pure solution under the optimum conditions of pH and ionic strength given in Table 2. The proposed method could be applied over a concentration range of 5.60 to 56.80 mg per25 ml solution of Rutin. The results revealed that, for $[Co(NH_3)_5Cl]^{2+}$, $[Cu(NH_3)_4]^{2+}$ and $[Cd(NH_3)_4]^{2+}$, recoveries are in the range of 100.21% to 101.10% (Table 3). The low values of relative standard deviation (0.91-1.16%) reflect the precision and accuracy of the method. Validity of the proposed method as an accredited method for Rutin determination was checked by Student's t-test statistical analysis at 95% confidence limit. t- values of 0.5-2.7 were obtained indicating that the differences between the average contents are not significant. Comparing this method with published spectrophoto-metric methods using F test with 5 degrees of freedom gave F values of 1.85-3.00 [11,17].These values indicates that no significant difference between the proposed and published methods (Table 3). However, the proposed method shows higher accuracy and precision as indicated by the smaller values of t- test.

Determination of Rutin in Pharmaceutical Preparations

In pharmaceutical preparations, Rutin was precisely determined using the proposed method under the optimum conditions of pH and ionic strength given in Table 2. The recoveries are in the range of 100.07 to 101.15%. Low relative standard deviation values (0.86-1.12%) reflect high precision and accuracy of the method. The proposed method is applied over a wide concentration ranges of 9.50 to 48.00 and 7.50 to 52.50 mg per 25 ml solution for Ruta-C-60 and Rutin-C tablets, respectively. Evaluation of the proposed method for Rutin in Tablets was performed by applying Student's t-test and F-test statistical analysis. Student t-test at 95% confidence limit gave t- values of 0.48-2.99 indicating that the differences between the average contents of repeated measurements are not significant. The differences in precision between this method and published spectrophotometric method using F test with 5 degrees of freedom gave F values of 1.70-3.09 [11,17]. These values indicate with 95% confidence limit that, no significant difference between the proposed and published methods. The proposed method show higher accuracy and precision as indicated by the low t-values which means that no systematic differences between the determined and true concentration over the cited ranges.

Specificity, Interference, Selectivity

Owing to the fact that, formation of ion associates requires the existence of negatively charged species produced only by hydrolysis of Rutin, makes this reaction specific for Rutin. Other ingredients in the media will not interfere such reaction. Therefore, no extraction was required to separate Rutin from the drug matrix. Also the associate complex is precipitated from aqueous-methanol media and separated from other matrix ingredients. Selectivity towards excepiences and filler added to the pharmaceutical preparations is an important factor in pharmaceutical analysis. The results so far showed that fortunately, these substances do not interfere and the reaction used is selective towards Rutin (Table 3).

Precision

The mean standard deviation for five determinations are ≤ 1.20. This level of precision is quite suitable for quality control analysis of pharmaceutical preparations and natural products [11].

Conclusion

AAS has the advantages of being fast and simple compared to other analytical techniques. The most exciting advantage for AAS is the high sensitivity, low detection limit and absence of interference in our case. This makes this method exhibits fair sensitivity, accuracy and reproducibility superior to that obtained by other methods in the literature.

The proposed method could be successfully used for determining Rutin in its pure and pharmaceutical dosage forms. The method has proved to be simple, sensitive and precise (R.S.D= 0.91-1.16% and 0.86-1.12% for pure and pharmaceutical dosage forms, respectively). It can be carried out without pretreatment of pharmaceutical samples. It also has a high degree of specificity and could be applied over wide working ranges of, 5.6-56.8 mg, 9.50 to 48.00 and 7.50 to 52.50 mg per 25 ml solution for pure Rutin, Ruta-C-60 and Rutin-C tablets, respectively. Therefore, this method is valid for Rutin determination and can be accredited for routine control analysis of this drug and similar drug compounds.

Table 1. Composition, m.p °C, molar ratio (Ion:rutin), color and elemental analysis of the ion associate complexes.

Ion Assoc. Composition	m.p.	M.R.	Color	Calculated/(Found)			
				C%	H%	N%	M%
$[Co(NH_3)_5Cl](C_{15}H_9O_7)_2$	215.0	1:2	Green	46.08	4.35	8.98	7.53
				(47.16)	(4.15)	(9.36)	(7.89)
$[Cu(NH_3)_4](C_{15}H_9O_7)_2$	276.0	1:2	Green	49.09	4.12	7.63	8.66
				(51.06)	(4.00)	(8.00)	(9.07)
$[Cd(NH_3)_4](C_{15}H_9O_7)_2$	242.0	1:2	Green	46.02	3.86	7.16	14.36
				(48.10)	(3.74)	(7.48)	(15.02)

Table 2. Solubility (pS) and solubility product (pK_{sp}) of the ion associates at their optimum condition of pH and ionic strength (I) at 25°C.

Ion Associate	pH	I	pS	pK_{sp}
$[Co(NH_3)_5Cl](C_{15}H_9O_7)_2$	3.0	0.2	5.00	14.42
$[Cu(NH_3)_4](C_{15}H_9O_7)_2$	4.0	0.3	4.74	13.63
$[Cd(NH_3)_4](C_{15}H_9O_7)_2$	3.0	0.5	4.63	13.89

pS = -log (solubility), (S).

pK_{sp} = - log (solubility product), (K_{sp}).

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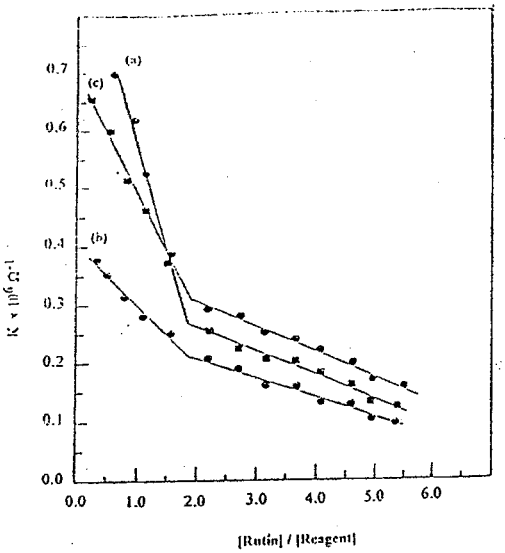

Fig. 1. Conductometric titration curves of 50 ml (1.6×10^{-4} M) Rutin in methyl alcohol with 10^{-3} M of (a) $[Co(NH_3)_5Cl](ClO_4)_2$, (b) $[Cu(NH_3)_4](ClO_4)_2$ and (c) $[Cd(NH_3)_4](ClO_4)_2$.

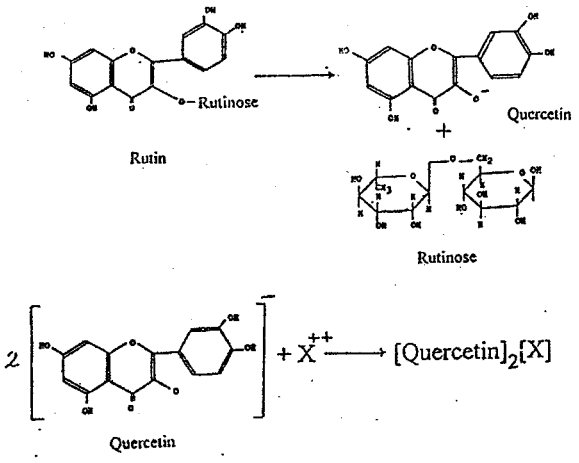

Fig. 2. Mechanism of the associate complexes formation from Rutin and the corresponding metal complex ion. X stands for $[Co(NH_3)_5Cl]^{2+}$, $[Cu(NH_3)_4]^{2+}$ or $[Cd(NH_3)_4]^{2+}$.

Table 3. Determination of Rutin in authentic samples and in pharmaceutical preparations by AAS.

Sample	Taken (μg)	Proposed Method			Reference Method [17]			F-test***
		recovery% (Mean)*	RSD% (Mean)*	t-test	recovery% (Mean)*	RSD% (Mean)*	t-test**	
using $[Co(NH_3)_5Cl]^{2+}$								
Rutin solution, authentic	5.60-56.80	101.10	0.91	2.70	104.5	0.67	7.51	1.85
Ruta-C-60, tablets(a)	9.50-48.00	100.40	1.03	0.87	97.4	0.66	4.41	2.44
Rutin-C, tablets(b)	7.50-52.50	101.12	1.16	2.16	97.4	0.66	4.41	3.09
using $[Cu(NH_3)_4]^{2+}$								
Rutin solution, authentic	5.60-56.80	101.07	0.98	2.44	104.5	0.67	7.51	2.14
Ruta-C-60, tablets	9.50-48.00	101.12	1.12	2.24	97.4	0.66	4.41	2.88
Rutin-C, tablets	7.50-52.50	101.15	0.86	2.99	97.4	0.66	4.41	1.70
using $[Cd(NH_3)_4]^{2+}$								
Rutin solution, authentic	5.60-56.80	100.21	1.16	0.50	104.5	0.67	7.51	3.00
Ruta-C-60, tablets	9.50-48.00	100.23	1.08	0.48	97.4	0.66	4.41	2.68
Rutin-C, tablets	7.50-52.50	100.56	1.04	1.20	97.4	0.66	4.41	2.48

*average of five determinations for the relative standard deviation (RSD%) and recovery%.

** calculated for the proposed and reference methods separately bu considering the real value in each case.

*** calculated by comparing the standard deviation of the proposed and reference methods.

(a)Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt

(b)PharcoPharm. Co., Alexandria, Egypt.

تقدير الروتين فى صورته النقية والدوائية باستخدام طريقة غير مباشرة لقياس الامتصاص الذرى

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تم اقتراح طريقة غير مباشرة لتقدير دواء الروتين فى صورته النقية وكذلك فى المركبات الصيدلية. تعتمد هذه الطريقة على ترسيب متراكبات الترابط الأيونى (Ion association complexes) بين الروتين وكل من $[Co(NH_3)_5Cl]^{2+}$, $[Cu(NH_3)_4]^{2+}$ و $[Cd(NH_3)_4]^{2+}$ حيث يتم تقدير الزيادة من ايونات النحاس والكوبالت غير المتفاعلة باستخدام قياس الامتصاص الذرى.

وقد تم بنجاح استخدام هذه الطريقة لتقدير دواء الروتين فى حالته النقية وكذلك فى الاشكال الصيدلية حيث تم تقدير الدواء فى مدى التركيز من 5.6 حتى 56.8 جزء لكل مليون جزء (ppm).بمتوسط انحراف معيارى نسبى من 0.91 الى 1.16% وقد تبين أن نسبة الناتج (Recovery %) تتراوح من 100.2 حتى 101.1% من القيمة الفعلية للروتين فى العينة المقاسة.

هذه النتائج توضح الدقة العالية ودرجة الاحكام فى التقدير للطريقة المقترحة وعند معاملة نتائج الطريقتين إحصائيا باستخدام اختبار t واختبار F ومقارنتها بطريقة تقدير منشورة أعطت نتائج بنسب تطابق جيدة ونسبة ثقة تصل الى ٩٥%.

هذه النتائج توضح امكانية استخدام هذه الطريقة الجديدة للتحاليل الدورية لرقابة الجودة لهذا الدواء والادوية المتشابهة.