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Spectrophotometric Determination of Tiopronin in Pharmaceutical Preparations

قياس تركيز مركب التيوبرونين في المستحضرات الدوائية باستخدام جهاز المطياف الضوئي

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Abstract

Two spectrophotometric methods are described for the determination of tiopronin in pharmaceuticals. They are based on the oxidation-reduction reaction between tiopronin and iron (III), then forming a complex between iron (II) and ferrozine or di-2-pyridyl ketone-2-thiophenoylhydrazone. The produced colored iron (II)-ferrozine complex [system I] absorbs at 562 nm, while the iron (II)-di-2-pyridyl ketone-2-thiophenoylhydrazone complex [system II] absorbs at 656 nm. The effect of different factors such as: pH, reagent concentration, time of reaction, temperature and the tolerance amount of the common excipients have been studied. Applying the optimum working conditions, tiopronin can be determined over the range 0.2-8.6 and 0.5-17.0 ppm and with molar absorptivities of 2.0×10^4 and 1.0×10^4 $l\ mol^{-1}cm^{-1}$ for systems I and II, respectively. Both methods offer high selectivity, sensitivity and accuracy with a relative standard deviation (RSD) of less than 1.1% for five measurements. The proposed methods were applied successfully for the determination of tiopronin in Captimer tablets.

Key Words: Spectrophotometry, Tiopronin, Ferrozine, Di-2-pyridyl ketone-2-Thiophenoylhydrazone (DPKTH), Pharmaceutical analysis.

ملخص

تتضمن المخطوطة طريقتين تعتمدان على تفاعل التأكسد والاختزال بين مركب التيوبرونين وأيونات الحديديك لتكوين معقد الحديديك/فيروزين (الطريقة الأولى) أو تكوين معقد الحديديك/ ثنائي-٢- بيرديدل كيتون-٢- ثيوفينيل هيدرازون المعقد (الطريقة الثانية). في كلا الطريقتين يقوم مركب التيوبرونين باختزال أيون الحديديك إلى أيون الحديدوز والذي بدوره يكون مع الفيروزين المركب المعقد الذي يمتص عند طول موجه مقدارها

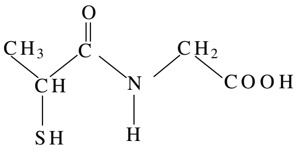
٥٦٢ نانوميتر. بينما يكون أيون الحديدوز مع ثنائي -٢ - بيرديدل كيتون -٢ -ثيوفينيل هيدرازون المركب المعقد الذي يمتص عند طول موجه مقدارها ٦٥٦ نانوميتر.

لقد تمت دراسة العوامل التي تؤثر على تفاعل التأكسد والاختزال وعلى تكوين المركبين المعقدين المذكورين المعقدان المذكوران و اللازمة لإعطاء أعلى قيمه امتصاصية. ومن العوامل التي تمت دراستها: درجه الحموضة, تركيز المواد المتفاعلة, زمن التفاعل, درجه الحرارة وتأثير المواد الأخرى التي تستعمل في تركيب المستحضرات الدوائية. وقد دلت نتائج البحث أن المنحنى القياسي لتركيز التيوبرونين أعطى علاقة خطيه محصورة بين ٠,٢ -٨,٦ جزء في المليون باستخدام الطريقة الأولى و٠,٥ -١٧,٠ جزء في المليون باستخدام الطريقة الثانية, وكانت قيم ثابت الامتصاص ألجزيئي ٢,٠x ١٠ ٤ و ١,٠x ١٠ ٤ لتر مول-١سم-١ باستخدام الطريق الأولى والثانية, على التوالي. لقد تم تطبيق الطريقتين المذكورتين وبنجاح في قياس تركيز مركب التيوبرونين في أقراص ألادويه التي تحمل اسم كابتمير.

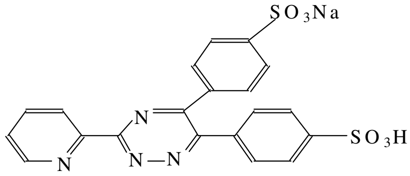
Introduction

Tiopronin (N-2-mercaptopropionylglycine), is a therapeutic agent used in the treatment of some hepatic and skin disorders, cystinuria, rheumatoid arthritis, and heavy metal poisoning [1-2]. Due to its presence in biological materials and pharmaceutical preparations, several methods for its determination have been reported in the literature including flow injection [3-8], liquid chromatography [9], gas chromatography - mass spectrometry [10], voltammetry [11], high-performance liquid chromatography [12-16], titrimetry [17] and spectrophotometry [18-20].

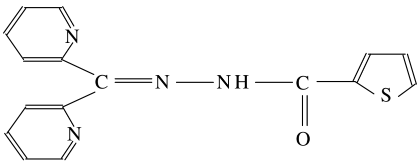
In the present work two new methods are proposed for spectrophotometric determination of tiopronin. They are based on the oxidation-reduction reaction of tiopronin with iron(III)-ferrozine complex [system I] to produce iron (II)-ferrozine complex which absorbs at 562 nm, or with iron (III)-di-2-pyridyl ketone-2-thiophenoylhydrazone complex [system II] to produce iron(II)-di-2-pyridyl ketone-2-thiophenoylhydrazone complex which absorbs at 656 nm.



Tiopronin



Ferrozine



DPKTH

Experimental

Reagents and Solutions

Inorganic chemicals were all of analytical grade.

Ferrozine [3-(2-pyridyl)-5,6-bis (4-phenylsulfonic acid)-1,2,4-triazine] (Fz) was used as purchased from Aldrich.

Di-2-pyridyl ketone-2-thiophenoylhydrazone (DPKTH) was prepared as described earlier [21]. Stock reagent solutions (1.0×10^{-2} M) was prepared by dissolving the appropriate amounts in known volumes of ethanol.

Tiopronin was purchased from Sigma and was used as working standard.

Britton and Robinson buffers in the pH range 2-11 were prepared from boric acid, phosphoric and acetic acid and sodium hydroxide.

Apparatus

A Unicam UV/vis spectrophotometer UV2 with 1 x 1-cm quartz cell was used for recording spectra and absorbance measurements.

General Procedure

Determination of Tiopronin Using System I

A portion of solution containing tiopronin in the range 2.0-86.0 μg was transferred into a 10-mL volumetric flask, then 0.5 mL of 1.0×10^{-3} M ferric chloride solution was added followed by the addition of 0.3 mL of 1.0×10^{-2} M ferrozine and 4.0 mL of 0.25 M KNO_3 solutions, respectively. Finally the volume was completed to 10 mL with buffer (pH = 5.0). After 1 minute, the absorbance was measured at 562 nm against water as a blank in a thermostated bath at 20°C.

Determination of Tiopronin Using System II

A portion of solution containing tiopronin in the range 5.0-170.0 μg was transferred into a 10-mL volumetric flask, then 0.5 mL of 1.0×10^{-3} M ferric chloride solution was added followed by 0.3 mL of 1.0×10^{-2} M DPKTH and 4.0 mL of 0.25M KNO_3 solution. The volume was completed to 10 mL with buffer (pH = 6.0). After 1 minute, the absorbance was measured at 656 nm against water as a blank in a thermostated bath at 20°C.

Determination of Tiopronin in Captimer Tablets

Captimer (commercially available as tablets from Fresenins AG, 61343 Bad Hamburg V.D.H). A tablet containing 100 mg of tiopronin was dissolved in water and the volume was completed to exactly 500 mL. After stirring, the solution was filtered, and the above procedures were followed for the spectrophotometric determination of tiopronin.

Results and Discussions

The proposed methods are based on the ability of tiopronin to reduce iron (III) to iron (II) which is rapidly converted in the presence of ferrozine or DPKTH to the highly stable colored complexes $Fe(Fz)_3^{2+}$ [system I] [22] or $Fe(DPKTH)_2^{2+}$ [system II] which absorb at 562 and 656 nm, respectively. To find the optimum analytical conditions for the determination of tiopronin, the dependence of absorbance on time of reaction, pH, ionic strength, temperature and ferric ion concentration were investigated.

Absorption Spectra

The absorption spectra of the colored complexes Fe $(Fz)_3^{2+}$ [system I] and $Fe(DPKTH)^{2+}$ [system II] were studied in the wavelength range 400-800 nm for solutions prepared as described in the general procedure. The obtained results showed that system I has maximum absorbance at 562 nm, while system II has maximum absorbance at 656 nm.

Effect of Time of Reaction

The effect of time of reaction on the absorbance was studied for both systems. The obtained results showed that maximum color intensity of the iron (II)-complexes was attained after one minute of the addition of the ligand and the intensity remains constant for at least 24 hours.

Effect of pH

The effect of pH on absorbance was studied in the range 2-11 for both systems. Maximum absorbance was obtained in the pH ranges 4.5-5.5 and 6.0-6.5, when using the ligands ferrozine and DPKTH, respectively. The obtained results are presented in Figure 1.

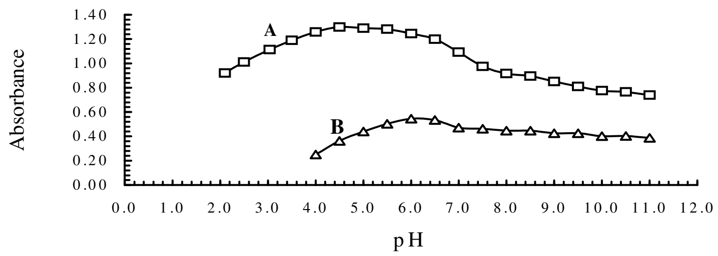


Figure 1: Effect of pH on absorbance
A: For iron (II)-Fz system (I) at 562 nm.
B: For iron (II)-DPKTH system (II) at 656 nm
Conditions: $[Fe^{+3}]=5.0x10^{-5}$ M, $[Fz]=3.0x10^{-4}$ M, $[DPKTH]= 3.0x10^{-4}$ M, $[Tiopronin]=1.0x10^{-4}$ M. Temperature = 20°C.

Effect of Temperature

The effect of temperature on the absorbances for both systems was studied in the range 20-45°C. The obtained results showed that maximum absorbance occurs at 20°C. Any further increase in the temperature showed a gradual decrease in the absorbance. In the present work, all spectrophotometric measurements were carried out in a thermostated bath at 20°C. The obtained results are presented in Figure 2.

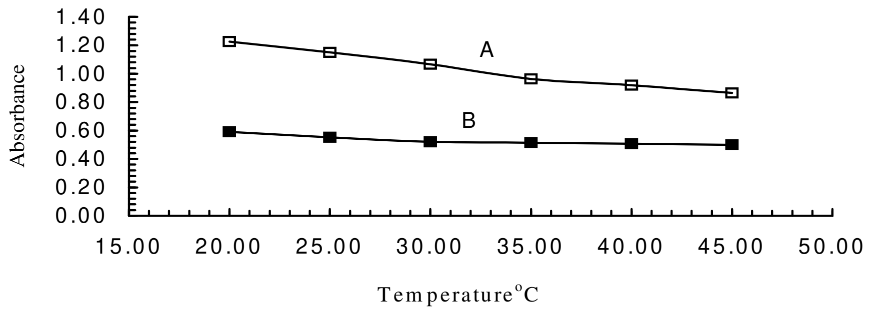


Figure 2: Effect of temperature on absorbance
A: For iron (II)-Fz system (I) at 562 nm and pH 5.0
B: For iron (II)-DPKTH system (II) at 656 nm and pH 6.0
Conditions: $[Fe^{+3}]=5.0x10^{-5}$ M, $[Fz]=3.0x10^{-4}$ M, $[DPKTH]= 3.0x10^{-4}$ M, $[Tiopronin]=1.0x10^{-4}$ M.

Effect of Iron (III) Concentration

The effect of iron (III) concentration on the absorbance of both systems was studied. It was found that keeping tiopronin and the ligand (ferrozine or DPKTH) at constant concentration and increasing the concentration of iron (III) resulted in an increase in the absorbance up to an iron(III):ferrozine molar ratio of 1:3 and an iron(III):DPKTH molar ratio of 1:2. Any further increase in the concentration of iron(III) did not show any effect on the absorbances of both systems up to a molar ratio of 2:1 as shown in Figure 3. On the other hand, the iron (III):tiopronin molar ratio was found to be 2:1 in both systems as shown in Figure 4.

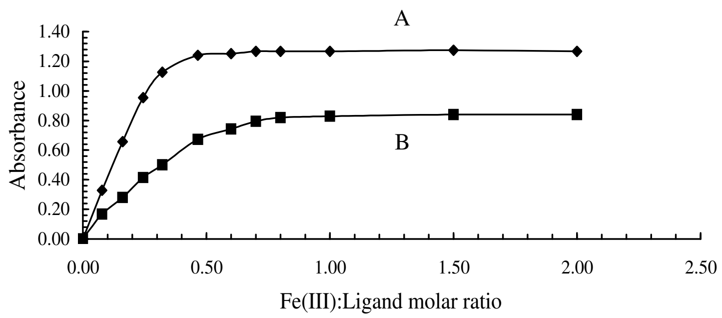


Figure 3: A: Iron (III): Fz molar ratio at 562 nm and pH 5.0, [Fz]=$2.0x10^{-5}$ M, [Tiopronin]=$1.0x10^{-4}$ M.
B: Iron (III): DPKTH molar ratio at 656 nm and pH 6.0, [DPKTH]= $2.0x10^{-4}$ M, [Tiopronin]=$1.0x10^{-4}$ M.

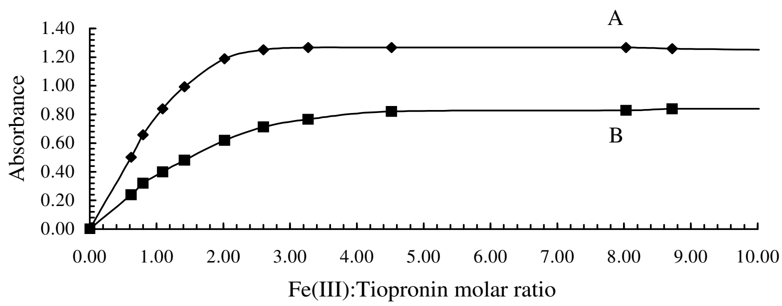


Figure 4: Iron (III): Tiopronin molar ratio
A: System I at 562 nm and pH 5.0, [Fz]=$2.0x10^{-3}$ M, [Tiopronin]=$1.0x10^{-4}$ M.
B: System II at 656 nm and pH 6.0, [DPKTH]= $2.0x10^{-3}$ M, [Tiopronin]=$1.0x10^{-4}$ M.

Effect of Potassium Nitrate Concentration

The effect of ionic strength on the oxidation rate of tiopronin by the iron (III) was studied through the addition of potassium nitrate and measurement of absorbance for both systems. The results showed that for both systems the rates of oxidation decrease slowly with the increase in potassium nitrate concentration. A concentration of 0.1M was selected as the optimum concentration. At this concentration, the complexes are stable and their solubilities are high, since it was found that without adding KNO_3 solution, the resulted iron (II)-DPKTH complex precipitated after few minutes from its solution. Addition of potassium nitrate kept the complex in solution and prevent its precipitation.

Interference Studies

Interference by foreign species was studied for the determination of tiopronin using both systems. Since the aim of this work is the determination of the tiopronin in pharmaceutical formulation, the effect of the common tablet fillers was especially considered. The obtained results are presented in Table 1. The proposed methods are selective since slight interference due to excipients has been observed when the additive to tiopronin mass ratio does not exceed 500:1.

Calibration Curves

Following the general procedures, linear relationships were obtained between the absorbance and the concentration of tiopronin within the range 0.2-8.6 and 0.5-17.0 ppm when using ferrozine system (I) and DPKTH system (II), respectively. The detection limits were found to be 0.2 and 0.5 ppm when using system I and system II, respectively. The sensitivity of the method for the determination of tiopronin expressed in terms of molar absorptivity is 2.0×10^4 and 1.0×10^4 $l\ mol^{-1}cm^{-1}$ for the ferrozine system (I) and DPKTH system (II), respectively. The precision of the proposed methods was checked by five successive measurements of 5.0×10^{-5} M tiopronin expressed as the relative standard deviations were found to be 0.84% and 1.1% for the ferrozine system (I) and DPKTH system (II), respectively.

Table 1: Recoveries of tiopronin in the presence of various additives used as excipients using ferrozine [system I] and DPKTH [system II].

Additive	Ferrozine [system I] [a]		DPKTH [system II] [b]	
	Additive: tiopronin Mass ratio	Recovery (%)	Additive: tiopronin Mass ratio	Recovery (%)
Sucrose	500:1	95.0	500:1	95.7
	1000:1	92.5	1000:1	91.8
Lactose	500:1	97.0	500:1	96.4
	1000:1	94.4	1000:1	93.8
Magnesium stearate	500:1	95.5	500:1	93.4
	1000:1	92.0	1000:1	89.2
Sodium bicarbonate	500:1	92.0	500:1	107.1
Carboxy methyl cellulose	500:1	96.3	500:1	97.2
	1000:1	92.0	1000:1	94.0
Starch	500:1	94.3	500:1	95.1
	1000:1	91.0	1000:1	91.9
Gum	500:1	95.0	500:1	96.3
	1000:1	91.0	1000:1	92.0
Talc	500:1	94.1	500:1	97.8
	1000:1	91.0	1000:1	94.3
K_2SO_4	500:1	101.2	500:1	102.5
	1000:1	104.9	1000:1	107.0
NaCl	500:1	950	500:1	107.0

a: [tiopronin]= $4.0x10^{-5}$ M, [Fe^{+3}]=$1.0x10^{-4}$ M, [Ferrozine]= $3.0x10^{-4}$ M, pH = 5.0.

b: [tiopronin]= $5.0x10^{-5}$ M, [Fe^{+3}]=$2.0x10^{-4}$ M, [DPKTH]= $4.0x10^{-4}$ M, pH = 6.0.

Determination of Tiopronin in a Pharmaceutical Formulation

The validity of the proposed methods for spectrophotometric determination of tiopronin was checked by the analysis of tiopronin in Captimer tablets (100 mg tiopronin per tablet). Recoveries in the range of 101.0-101.5% were obtained. The results are shown in Table 2.

Table 2: Determination of tiopronin in a pharmaceutical formulation.

Drug	Nominal Composition	Determined content as percentage of label claim ± standard deviation*	
		System I	System II
Captimer	100 mg tiopronin per tablet	101.5 ± 1.0	101.0 ± 1.1

* Average of five separate determinations.

Conclusion

Comparison between different methods for the determination of tiopronin (Table 3) indicates that the proposed two methods can be used as alternative methods for spectrophotometric determination of tiopronin with reasonable sensitivity, selectivity and accuracy. On the other hand, a comparison study between the two proposed methods of analysis (system I and system II) showed that both methods can be successfully used for determination of tiopronin in pharmaceutical preparations. Both methods proved to be sensitive, accurate and precise. The advantage of the method using Ferrozine lies in its higher sensitivity (molar absorptivity of 2.0×10^4) and precision (RSD 0.84%), and lower detection limit (0.2 ppm), while the advantage of the method using DPKTH lies in its large range of calibration curve.

Table 3: Comparison between different methods used for determination of tiopronin.

Technique	Reagent used	Linear Range or detection limit/ppm	RSD %	Reference
Flow-injection	Cerium(IV)+rhodamine 6G+quinine	0.02-11.5	2.6	3
	Cerium (IV) + quinine	0.16-65.0	2	4
	Tetrabutylammonium bromide/cetrimonium bromide	0.16-32.0	-	5
	Thallium (III)	0.13-3.2	1.0	6
	Lead chloride	1.6-98.0	0.3	7
	ClO^-+luminol	16.0-16300	-	8
Liquid Chromatography	Cobalt phthalocyanine	0.8-16.3	-	9
Gas chromatography	Acrylic acid esters	0.001	-	10
Voltammetry	-	$1.6X10^{-4}$-0.05	-	11
HPLC	Pyrene maleimide	0.2-10.0	6.2	12
	2,4-dinitrofluorobenzene	0.1-2.0	-	13
	N,N-(dimethylamino-4-methylcoumarine-3-yl) maleimide	0-16.3	3.0	15
	N,N-(dimethylamino-4-methylcoumarine-3-yl) maleimide	0-20.4	5.8	16
Titrimetry	$AgNO_3$	8.3-321	3.2	17

... *Table (3) Continued*

Technique	Reagent used	Linear Range or detection limit/ppm	RSD %	Reference
Spectrophotometry	Ammonium tetrachloropalladate	50.0-147.0	-	18
	5,5′-dithiobis-(2-nitrobenzoic acid)	1.0-6.0	-	19
	Iron(II)/ferrozine system (I)	0.2-8.6	0.84	Present work
	Iron(II)/DPKTH system (II)	0.5-17.0	1.1	Present work

References

1] Suzuki A., Kajimoto N., Yanagama T., Sugimoto J., Nagata M., *J. Toxical. Sci.*, **3** (1978) 313.

2] Basinger M., Gasas J., Jones M., Weaver A., Weinstein N., *J. Inorg. Nucl. Chem.*, **43** (1981) 1419.

3] Perez-Ruzi T., Martinez-Lozano C., Baeyens WRG., Sanz A., San-Miguel MT., *J. Pharm. Biomed. Anal.*, **17** (1998) 823.

4] Zhao Y.N., Baeyens WRG., Zhaug X.R., Calokerions AC., Nakashima K., Van-Der-Weken G., *Analyst*, **122** (1997) 103.

5] Zhao Y., Baeynes WRG., Zhang XR., Calokerinis AC., Nakashima K., Van-Der-Weken G., Van-Overbeke A., *Chromatographia*, **44** (1997) 31.

6] Perez-Ruiz T., Martinez-Lotinez C., Tomas V., Sidrach-De-Cardona C. *J. Pharm. Biomed. Anal.*, **15** (1996) 33.

7] Garcia MS., Sanchez-Pedreno C., Albero MI., Rodenas V., *J. Pharm. Biomed. Anal.*, **11** (1993) 633.

8] Lopez-Garcia I., Vinas P., Martinez-Gil JA., Fresenius. *J. Anal. Chem.*, **345** (1993) 723.

9] Favora G., Fiorani., *Anal Chim. Acta*., **332** (1996) 249.

10] Matsuura K., Takashina H., *J. Chromatogr, Biomed.Appl*., **127** (1993) 229.

11] Casassas E., Arino C., Esteban M., Redondo A., *Anal. Lett*., **24** (1991) 1183.

12] Leroy P., Nicolas A., Gavriloff C., Matt M., Netter P., Bannwarth B., Hercelin B., Mazza M., *J. Chromatogr. Biomed. Appl*., **102** (1991) 258.

13] Marzo A., Martelli EA., Bruno., Nava D., Mignot A., Vidal R., Lefebure MA., *J. Chromatogr*., **536** (1991) 327.

14] Cavrini V., Gatti R., Dipietra AM., Raggi MA., *Chromatographia*, **23** (1987) 680.

15] Kagedal B., Andersson T., Carlsson M., Denneberg T., Hoppe A., *J. Chromatogr. Biomed. Appl*., **61** (1987) 261.

16] Kagedal B., Calsson M., Dennberg T., *J. Chromatogr. Biomed. Appl*., **53** (1986) 301.

17] Vinas P., Cordoba MH., Sanchez-Pedreno C., *Analyst*, **115** (1990) 757.

18] Raggi M., Nobile L., Cavrini V., Di-Pietra AM., *Boll. C. F*., 1**25** (1986) 295.

19] Raggi M., Cesaroni M., Di-Pietra A., *Farmco*, **38** (1983) 312.

20] Raggi MA., Cavrini V., Di-Pietra AM., *J. Pharm. Sci*., **71** (1982) 1384.

21] Al-Nuri M., Abu-Eid M., Zatar N., Khalaf S., Hannoun M., Khamis M., *Analytica Chim. Acta*, **259** (1992) 175.

22] Stookey L., *Anal. Chem*, **42** (1970) 779.