Synthesis and Characterization of Tb(III)-acetylacetone complex and its analytical application for hydrochlorothiazide determination in pharmaceutical preparation and biological fluids

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Abstract

Tb(III)-Acetylacetone complex was prepared and characterized by elemental analysis, UV/Vis, FTIR, ^{1}H-NMR spectroscopy, mass spectroscopy, conductance and magnetism. The results indicated that the complex composition is $[Tb(ACAC)_2(NO_3)(EtOH)_2(H_2O)_2]$. The prepared complex was used as optical sensor for hydrochlorothiazide determination in pharmaceutical tablets and biological fluids (serum and urine). The hydrochlorothiazide can remarkably enhance the luminescence intensity of the complex in DMSO at $\lambda_{ex/em}$ = 285/545 nm and pH 6.3. The dynamic ranges found for the determination of hydrochlorothiazide concentration are 3.6 x 10^{-9} to 4.0 x 10^{-6} mol L^{-1}, and the limit of detection (LOD) and quantitation limit of detection (LOQ) are 1.3 ×10^{-9} and 4.2 x 10^{-9} mol L^{-1}, respectively.

Keywords: Tb(ACAC); Optical Sensor; Luminescence Intensity; Enhancement; Hydrochlorothiazide .

1. Introduction

Hydrochlorothiazide (6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide) (HCTZ) Fig.1. Is a diuretic medication often used to treat high blood pressure and swelling due to fluid buildup **[Vonaparti A., *et al.*, (2006); The American Society of Health-System Pharmacists, (2015)]**.

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Other uses include diabetes insipidus, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine **[The American Society of Health-System Pharmacists, (2015)]**.

It is in the thiazide medication class and acts by decreasing the kidneys' ability to retain water **[The American Society of Health-System Pharmacists, (2015)]**. This initially reduces blood volume, decreasing blood return to the heart and thus cardiac output **[Duarte, J. D., and Cooper-DeHoff, R. M., (2010)].** Long term, however, it is believed to lower peripheral vascular resistance **[Duarte, J. D., and Cooper-DeHoff, R. M., (2010)]** .

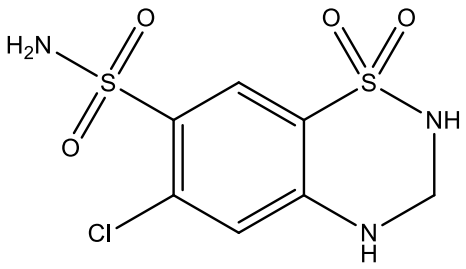

Fig. 1. Chemical structure of Hydrochlorothiazide

Many analytical methods are used to determine hydrochlorothiazide in pharmaceuticals and biological samples [**Ruiz-Angel, M. J., et al., (2006); Shaikh, B., (1996)].** These methods include spectrophotometry **[British Pharmacopoeia, (1996); Hegazy, M. A., et al., (2010)]**, Amperometric method **[Qingjiang Q. W., et al., (2003)]**, electrochemical method [**Rezaei, B., and Damiri, S., (2003)],** chromatography methods **[Niopas, I., and Daftsios, A. C., (2002); Zendelovska, D., et al., (2004); Vonaparti, A., et al., (2006)].** However, many of these methods are often time-consuming, technically demanding. So, there is an important demand for simple, low-cost, sensitive and rapid alternative method for the determination of hydrochlorothiazide in pharmaceuticals and biological samples.

The Terbium complexes have attracted more attention, due to their saturated red emission resulting from emitting strong fluorescence arising from f–f hyper sensitive transition with a large Stokes shift (approx. 250 nm) and long lifetime (approx. several hundred ms) **[Yuan, J., and Matsumoto, K., (1996)].** These distinct properties enabled the development of highly sensitive fluorescence chemical sensor. The intra-configuration 4f–4f transitions in rare earth ions are parity forbidden (Laporte rule), consequently the absorption and emission spectra of the Tb(III) ions show weak intensity. However, the population of the excited states of the Tb(III) ions may increase by coordination to organic ligands, which act as sensitizers. The ligands that present this property were

called by Lehn as "antennas" **[Azab, H. A., et al., (2010)].** In Tb(III)-complex, the organic ligand absorbs and transfers energy efficiently to the metal ion (intra- molecular energy transfer) and consequently increases its luminescence intensity.

In this paper the Tb(III)-acetylacetone complex was prepared and characterized using different spectroscopic techniques. The complex was used as optical sensor for determination of hydrochlorothiazide in pharmaceuticals and biological samples (serum and urine).

2.Material and Methods

2.1 Chemicals and reagents

All chemicals used were of analytical reagents grade obtained from Aldrich Chemical Company (USA). The drug standard (hydrochlorothiazide) was obtained from Sigma-Aldrich. The pharmaceutical preparations containing the drugs obtained from local drug stores. Urine and serum samples were obtained from healthy volunteers during morning hours.

2.2 Instruments

Elemental analyses carried out in Cairo University, Egypt. The IR spectra of the ligand and solid complex were recorded as KBr discs using JASCO FT/IR-460 infrared spectrophotometer. The electronic spectra (200-900nm) were carried out using a Perkin-Elmer 550 spectrophotometer. The ^{1}H-NMR spectra in deutrated dimethylsulfoxid (DMSO) as a solvent and were recorded on Gemini-300 MHz NMR spectrometer. Mass spectra of the solid complexes were recorded using Thermo Scientific, ISQ Single Quadrupole MS. The molar conductance of 10^{-3} M solution of metal complex in DMSO was measured on a dip cell and a Bibby conductimeter MC1 conductivity meter model. A magnetic measurement of the solid complex was measured at room temperature using Gouy's method by a magnetic susceptibility balance from Johnson Metthey and Sherwood model. The fluorescence measurements were carried out on a Shimadzu RF5301 spectrofluorophotometer in the range 290–750 nm.

2.3 methods

2.3.1. Preparation of the Tb(III)-Acetylacetone complex

Tb(III)-Acetylacetone complex, was synthesized by mixing 20 mL aliquot of 1×10^{-2} M of the ligand with a 10 mL aliquot of 1×10^{-2} M Tb(III) nitrate (2:1 ligand to metal molar ratio) with stirring. The mixture was refluxed at about 80^{o}C for two hours; then the mixture was cooled to 0^{o}C. The

resulting precipitate of the complex is yellowish white; the resulting precipitate of the complex was filtered off, and washed by ethylacetate**.**

To 10 mL clean measuring flasks, the standard solution of hydrochlorothiazide was prepared by different additions of 1 x 10^{-3} mol L^{-1} drug stock solution to give the following concentrations of the drug, 1 x 10^{-4} to 1 x 10^{-9} mol L^{-1}. The solutions were diluted to the mark with DMSO at room temperature. The above solutions were used for subsequent measurements of absorption and emission spectra as well as the effect of solvents and pH. The fluorescence intensities were measured at $\lambda_{ex}/\lambda_{em}$ =285/545.

2.3.2. Determination of hydrochlorothiazide in pharmaceutical preparations

Ten tablets of hydrochlorothiazide were carefully weighed and ground to finely divided powders. Accurate weights equivalent to 1.0 mg hydrochlorothiazide was dissolved in 50 mL DMSO and mixed well and filtered up using 12 mm filter papers. The concentration of the drug was determined by using different concentrations from the corresponding calibration graph.

2.3.3. Determination of hydrochlorothiazide in serum samples

A 1.0 mL of samples of blood collected from various healthy volunteers was centrifuged for 15 min at 4000 r/min to remove proteins. The unknown amount of drug in human serum samples was determined using the standard addition (spiking) techniques.

2.3.4. Determination of hydrochlorothiazide in urine samples

The urine samples studied, which were obtained from healthy male and female volunteers who had taken no drug previously, were processed in the laboratory as follows: 10 mL of urine were centrifuged for 15 min at 4000 r/min to remove precipitate for salts, crystals, pus cells, and red blood cells (RBCs). A 1.0 mL of urine was supplied with the volume of drug solutions. The unknown amount of drug in human urine samples was determined using the standard addition (spiking) techniques.

3.Result and Discussion

3.1 Characterizations of the Tb(III) Acetylacetone complex

The electronic absorption spectra of the ACAC ligand and complex were measured in ethanol at room temperature table 1. Uv/Vis spectra of the Tb(III) complex showed an intense high-energy absorption band at 224 nm. These high-energy absorption bands are assigned to $\pi - \pi^*$ transition in the complex **[Gusev, A.N., et al., (2013)].**

The electronic absorption band of the ligand was compared with that of the complex. We can observe that the band of the ligand, which appeared at 238 nm, shifted to 224 nm in the complex. This shift may be due to the complexation.

The IR spectrum of the complex was summarized in (table 1). The stretching band at 1637 cm^{-1} present in the ACAC spectrum was assigned to the C=O bond. This stretching band shifted to 1614 cm^{-1}. This shift confirmed also the participation of the carbonyl group in the complexation with Tb(III) ion **[Reddy, K. H., et al., (1997)].** The IR absorption bands appeared at 1176, 783 cm^{-1} for ACAC; result from the in-plane and out-of-plane vibrations of C-H bonds. These bands were shifted to 1150, 732 cm^{-1} by complexation. These changes could be attributed to the change in rigidity of the ligand ring upon coordination **[Gusev, A.N., et al., (2013)].** In complex a broad band appeared in the range 3200–3600 cm^{-1} assigned to the water molecules and/or to the OH stretching vibration of the ligands and the ethanol molecules present in the complex **[Narang, K.K., and Singh, V.P., (1996)].** The new bands at 413 cm^{-1} were observed in the complex was attributed to M-O bond in complex **[Refat, M. S., et al., (2014); Azab, H. A., et al., (2015); Attia, M. S., et al., (2015); Abd-Elzaher, M. M.,et al., (2012)].**

The ^{1}H-NMR spectra of the ACAC, and Tb(III)-complex were measured in DMSO-d_6 at room temperature. The chemical shift data are given in (table 1), but unfortunately we could not obtain good spectra for the complex which may be due to highly paramagnetic properties of the complex. This adds difficulty to assigning the NMR peaks.

Table 1. Important electronic absorption, IR, and ^{1}H-NMR data of the ligand and Tb(III)-complex.

Ligand/ Complex	*Absorption Bands(λ) nm*	*Important IR spectral data*				*^{1}H NMR (DMSO-d_6), δ in ppm*
		$\upsilon_{C=O}$	*δ_{C-H} (in plane)*	*δ_{C-H} (out of plane)*	*υ_{Eu-O}*	
ACAC Ligand	*238, 274*	*1637*	*1176*	*783*	*……*	*2.01 (S, 6H, $2CH_3$), 2.08 (S, 6 H, $2CH_3$), 3.80 (S, 2H, CH_2 -Keto form), 5.69 (S, H, CH-enol form), 15.61 (S, H, OH)*
Tb(III) Complex	*224*	*1614*	*1150*	*732*	*413*	*2.50 (m, H, CH3), 8.33 (m, H, CH)*

The molar conductivity of 1 x 10^{-3} M solution of the metal complex in DMSO at room temperature was found to be 13.96 ohm^{-1} cm^2 mol^{-1} (table 2) indicating that the complex is nonelectrolytic in nature **[Narang, K.K., and Singh, V.P., (1996); Refat, M. S., et al., (2014)].** The magnetic moment value of complex was measured using Gouy method and was found 9.62 B.M (table 2). The result indicated that Tb(III) complex was highly paramagnetic. The Tb(III) ions were paramagnetic due to their 4f-electrons that were effectively shielded by $5s^25p^6$ electrons **[Narang, K.K., and Singh, V.P., (1996)].**

Table 2. Conductivity, magnetism and elemental analysis of complex.

Complex	*Formula (formula weight)*	*Calcd. (found)*			*Am (Ω^{-1} cm^2 mol^{-1})*	*μ_{eff} (B.M.)*
		C	H	N		
Tb-ACAC Complex	*$C_{14}H_{34}NO_{11}Tb$ (551.35)*	*30.50 (30.59)*	*6.22 (6.00)*	*2.54 (2.19)*	*13.96*	*9.62*

The mass spectrum of Tb(III) complex reveals the molecular ion peak m/z at 551.35 a.m.u., consistent with the molecular weight of the structure of the complex shown in Fig. 2.

The elemental analysis of the complex is consistent with the calculated results from the empirical formula (table 2). The results indicated that the complex is ten-coordinated. On the basis of the physical and spectral data of the complex discussed above, one can deduce that the metal ions are bonded to two molecules of the ligand as well as one molecule of the nitrate ion and two molecules of water and two molecules of ethanol.

Complex may take the formula $[Tb(ACAC)_2(NO_3)(EtOH)_2(H_2O)_2]$, as illustrated in Fig. 3. **[Gusev, A.N., et al., (2013); Reddy, K. H., et al., (1997); Narang, K.K., and Singh, V.P., (1996); Refat, M. S., et al., (2014)].**

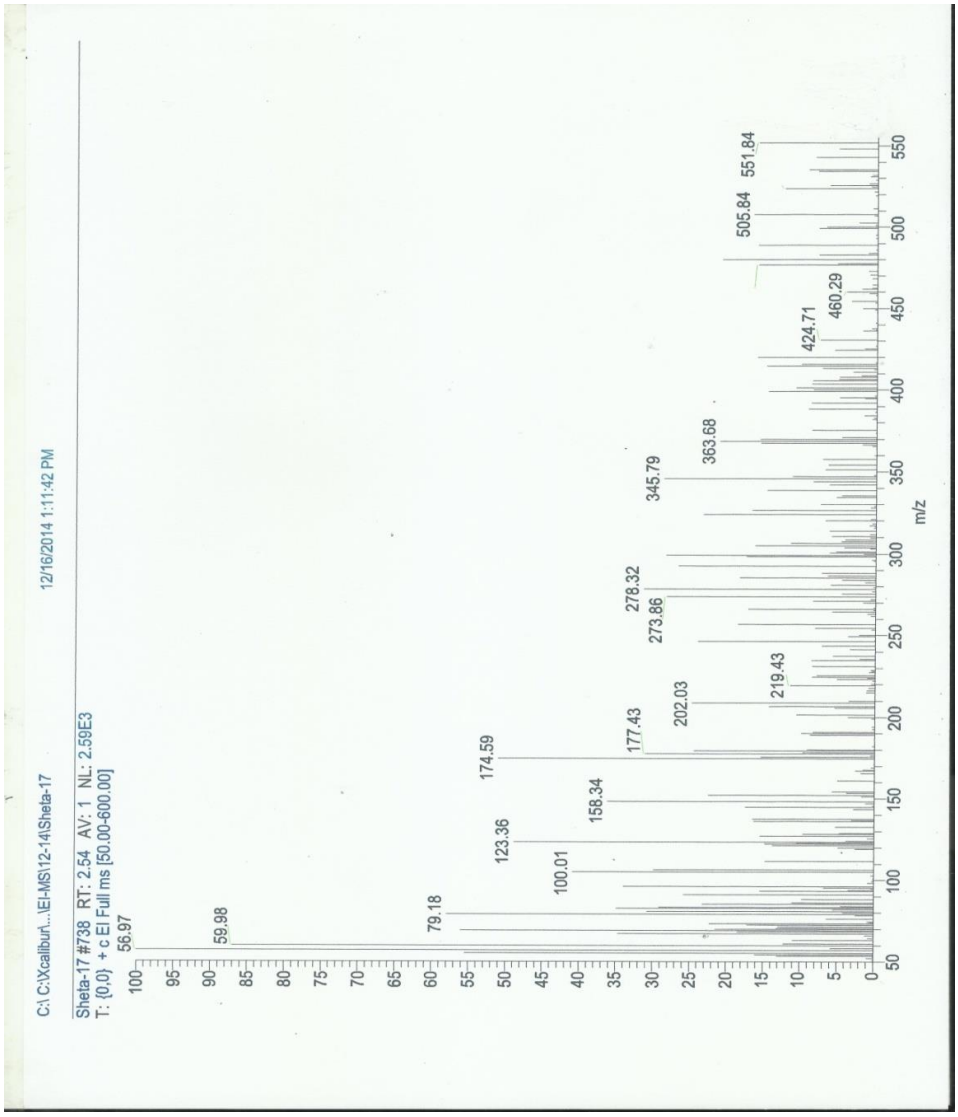

Fig.2. Mass Spectra of Tb(III) complex.

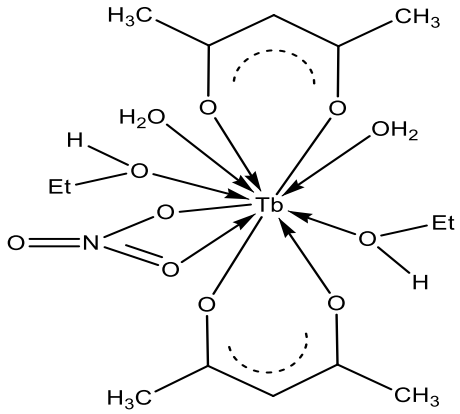

Fig. 3. Structural representation of complex .

3.2. Determination of hydrochlorothiazide using Tb(III) Complex by Spectrofluorimetric Method

3.2.1. Spectral Characteristics

3.2.1.1Absorption spectra

The absorption spectrum of ACAC is showed in Fig. 4. The band at 274 nm is attributed to π-π* transition. The absorbance is also enhanced, by increasing the concentration of the hydrochlorothiazide.

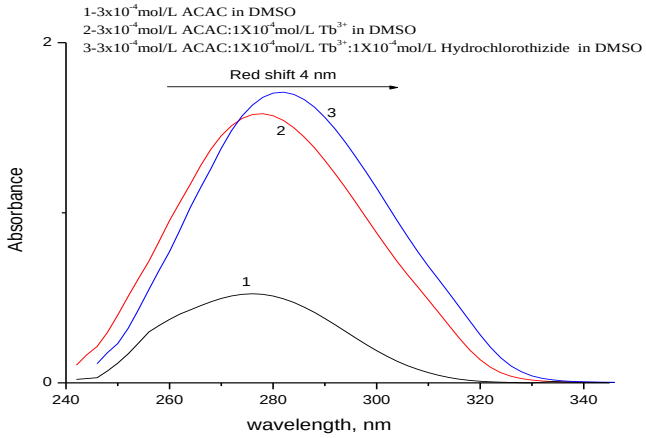

Fig. 4. The absorption spectra of the ACAC[1], Tb(III)-Acetylacetone[2], and Tb(III)-Acetylacetone with hydrochorothiazide[3].

3.2.1.2. Emission and excitation spectra

From the emission and excitation spectra of hydrochlorothiazide, Tb(III), complex Tb(III)-Acetylacetone and hydrochlorothiazide -Tb(III)-Acetylacetone, it can be seen that Tb(III) ion has two very weak peaks. Comparing of hydrochlorothiazide spectrum, after the addition of

hydrochlorothiazide into the Tb(III)-Acetylacetone, show that hydrochlorothiazide can form a complex with Tb(III)-Acetylacetone. Comparing spectra hydrochlorothiazide -Tb(III)-Acetylacetone with complex Tb(III)-Acetylacetone, it can be seen that the characteristic peak of Tb(III) at 545 nm has remarkably been enhanced after the addition of hydrochlorothiazide , which indicates that hydrochlorothiazide effectively enhance the energy of hydrochlorothiazide -Tb(III)-Acetylacetone complex.

3.2.2. Effect of experimental variables

3.2.2.1 Effect of pH

The pH of the medium has a great effect on the fluorescence intensity and absorption spectrum of the hydrochlorothiazide. The pH has been adjusted using NH_4OH and HCl. The optimum pH value where the peak 545 nm has the highest intensity was obtained at pH = 6.3.

3.2.2.2 Effect of Solvent

The influence of the solvent on the fluorescence intensity of the hydrochlorothiazide was measured in different solvents. The results show that there is no quenching in the emission intensity of hydrochlorothiazide in the presence of DMSO, see (Fig. 5).

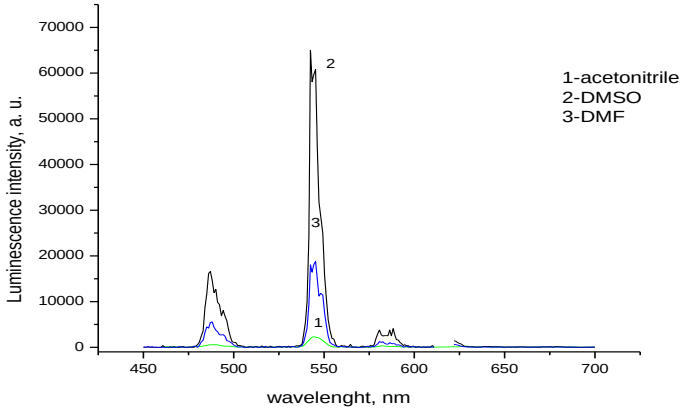

Fig. 5. The fluorescence spectra of the Tb(III)-Acetylacetone in different solvents, λ_{ex} = 285 nm.

3.2.2.3 Effect of hydrochlorothiazide Concentration

The influence of the amount of hydrochlorothiazide on the fluorescence intensities of the Tb(III)-Acetylacetone complex was studied. The emission spectra of the Tb(III)-Acetylacetone gives a characteristic band at 545 nm after excitation at 285 nm and the fluorescence intensity was enhanced by increasing the concentration of the hydrochlorothiazide till 1 x 10^{-4} mol L^{-1} then became constant. The experimental results showed that the fluorescence intensity reached maxima

and remained constant when hydrochlorothiazide concentrations are 1 x 10^{-4} mol L^{-1} in the DMSO preparations (Fig.6).

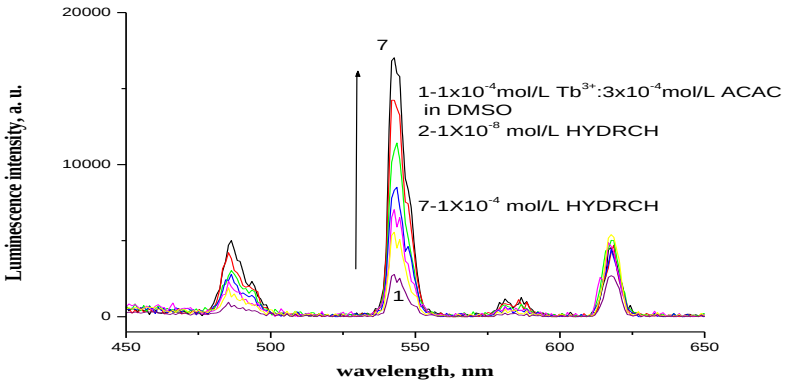

Fig. 6. The fluorescence spectra of the Tb(III)-Acetylacetone at different concentrations of hydrochlorothiazide in DMSO at λ_{ex} = 285 nm and pH 6.3.

3.2.3. Analytical Performance and Method Validation

3.2.3.1. Calibration curve

A linear correlation was found between fluorescence intensity of the Tb(III)-Acetylacetone complex at λ_{em} = 545 nm and concentration of hydrochlorothiazide in the ranges given in (table 3). The eleven-point 3.6 x 10^{-9} to 4.0 x 10^{-6} calibration curve was obtained and the graph was described by the regression equation:

$$Y = a + bX$$

(Where Y = fluorescence intensity of the sensor at λ_{em} =545 nm; a = intercept; b = slope and X = concentration in mol L^{-1}), (Fig. 7)

Regression analysis of hydrochlorothiazide intensity data using the method of least square was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in (table 3). The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines **[International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, (2005)]** using the formulae:

LOD = 3.3 S/b and LOQ = 10 S/b,

(Where S is the standard deviation of blank fluorescence intensity values, and b is the slope of the calibration plot) are also presented in (table 3). The low value of LOD indicates the high sensitivity of the proposed method if compared by the previous methods of the determination of hydrochlorothiazide see (table 4).

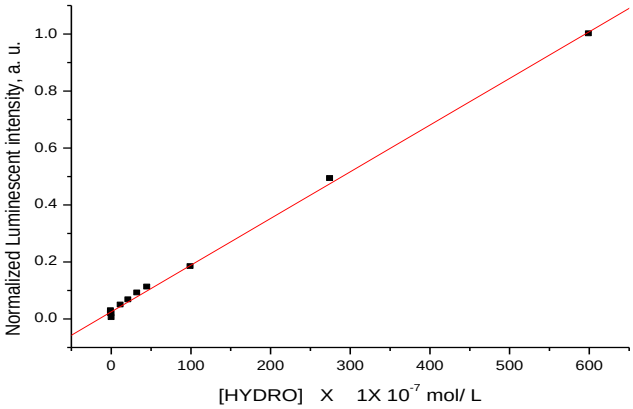

Fig. 7. linear relationship between concentration of hydrochlorothiazide and Normalized luminescence intensity of Tb(III)-Acetylacetone complex in DMSO.

Table 3. Sensitivity and regression parameters for the method.

Parameter	*Method*
λ_{em} *nm*	*545*
Linear range, mol L^{-1}	1×10^{-8} - 1×10^{-5}
Limit of detection (LOD), mol L^{-1}	4.5×10^{-9}
Limit of quantification (LOQ), mol L^{-1}	1.4×10^{-8}
*Regression equation, Y**	*Y=a+bX*
Intercept (a)	*0.024*
Slope (b)	0.002×10^{7}
Standard deviation	*0.012*
Variance (Sa2)x 10^{-4}	*1.4*
Regression coefficient (r)	*0.999*

*Where Y= fluorescence intensity, X= concentration in n mol L^{-1}, a= intercept, b= slope.

Table 4. Comparison of spectrofluorimetric technique with some existing methods for the determination of hydrochlorothiazide

Method	*Linear range*	*Detection limit*	*Ref.*
Amperometric method	$2.0x10^{-6}$ - $1.0x10^{-4}$	$1.0x10^{-6}$	***Qingjiang, Q. W., et al., (2003)***
LC-MS method	$1×10^{-9}$ –$1×10^{-4}$	$1×10^{-9}$	**Deventer, K., et al., (2002)**
HPLC method	$1×10^{-3}$ –$1×10^{-4}$	$1x10^{-5}$	**Ivanović.D, et al., (2007)**
Spectrofluorimetric method: HCTZ - Tb(III)-Acetylacetone	$3.6x\ 10^{-9}$ -$4.0\ x\ 10^{-6}$	1.3×10^{-9}	**The present work**

3.2.3.2. Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing talc (250 mg), starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (70 mg) and magnesium stearate (100 mg) was extracted with water and solution made as described under “analysis of dosage forms”. A convenient aliquot of solution was subjected to analysis according to the recommended procedures. In the method of analysis, there was no interference by the inactive ingredients.

A separate test was performed by applying the proposed method to the determination of hydrochlorothiazide in a synthetic mixture. To the placebo blank of similar composition, different amount of hydrochlorothiazide of different products were added, homogenized and the solution of the synthetic mixture was prepared as done under “analysis of dosage forms”. The filtrate was collected in a 100 ml flask. Five ml of the resulting solution was assayed (n=3) by proposed method which yielded a % recovery of 99.50 ± 0.65, 98.9 ± 1.75, and 97.60 ± 0.80 for tablet, urine, and serum samples, respectively. The results demonstrated the accuracy as well as the precision of the proposed methods. These results complement the findings of the placebo blank analysis with respect to selectivity.

3.2.3.3. Application to formulations

The proposed methods were applied to the determination of hydrochlorothiazide in Capozide tablets 25.0 mg ((Bristol-Myres Squib Com., Egypt) is purchased from local market and containing other inactive ingredients and in serum and urine samples of the health state human. The results show that the method is successful for the determination of hydrochlorothiazide and that the excipients in the dosage forms did not interfere. The results obtained were statistically compared with the official British Pharmacopoeia [B.P] method [**British Pharmacopoeia, (1999)],** and with those obtained by the United States Pharmacopeia method **[The United States Pharmacopeia, (2002)]**. The average recovery and R.S.D for the tablet, serum, and urine samples in our method found to be (100.2 **±** 1.43 %), (99.6 **±**0.70 %), and (103.1 **±** 1.70 %) respectively. Data obtained by B. P method average recovery (99.99 %, 98.92 and100.2.) for the tablet, serum, and urine samples respectively; and R.S.D 0.1 % were also presented for comparison and show a good correlation with those obtained by the proposed methods. The results obtained by the proposed methods agreed well with those of reference method and with the label claimed.

3.2.3.4. Recovery study

To further assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analysed tablet powder with pure hydrochlorothiazide at three different levels (0.1, 1.0 and 10.0 n mol L^{-1}) of the content present in the tablet powder (taken) and the total was found by the proposed method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between (99.75 and 102.09 %), (97.33 and 103.85 %), and (97.00 and 100.20 %) with relative standard deviation in the range (0.25 - 0.69 %), (0.61 - 0.85%), and (0.25 - 1.15%). for tablet, urine, and serum samples, respectively. Closeness of the results to 100 % showed the fairly good accuracy of the methods. The results are shown in (table 5).

Table 5. Results of recovery study using standard addition method.

Proposed method				
Tablet studied	***HCTZ in tablet extract, x 10^{-7} mol L^{-1}***	***Pure HCTZ added, x 10^{-7} mol L^{-1}***	***Total HCTZ found, x 10^{-7} mol L^{-1}***	***Pure HCTZ recovered (Percent±SD)***
Tablet	**10**	**0.5**	**0.40**	**99.75 ± 1.25**
	1.0	**1.0**	**0.95**	**98.75 ± 1.45**
	0.1	**1.5**	**1.65**	**102.09 ± 1.69**
Urine sample	**10**	**0.5**	**0.38**	**98.96 ± 0.81**
	1.0	**1.0**	**1.15**	**103.85 ± 0.55**
	0.1	**1.5**	**1.43**	**97.33 ± 0.67**
Serum sample	**10**	**0.5**	**0.46**	**99.65 ± 1.0**
	1.0	**1.0**	**0.92**	**97.0 ± 1.45**
	0.1	**1.5**	**1.61**	**100.2 ± 1.98**

4.Conclusion

The Tb(III)-Acetylacetone complex has high sensitive and characteristic peaks in the presence of hydrochlorothiazide . The intensities of these peaks are enhanced by increasing the concentration of hydrochlorothiazide, due to energy transfer from hydrochlorothiazide to the Terbium ion and can be used for determination of hydrochlorothiazide in pharmaceutical preparations and in biological fluids with high accuracy.

5.References

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الملخص العربى

اسماء المشاركون فى البحث:

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انتشرتعاطي واستخدام المركبات والعقاقير المنشطة بين كافه اطياف المجتمع الدولى والعربى خاصة, على سبيل المثال فى الوسط الرياضى بين الشباب انتشر تناول تلك العقاقير المنشطة بهدف زيادة الكفاءة والقدره البدنية وتحقيق الانجاز الرياضى السريع فى وقت قصير دون وعى وادراك لمخاطر تلك المركبات. ونظرا لاهمية هذا الموضوع عالميا فقد حذرت منظمة الصحة العالمية, و الوكالة الدولية لمكافحة المنشطات (الوادا) من استخدام وانتشار تلك العقاقير وفرض عقوبات صارمة دولية ومحلية على متعاطيها ومروجها, كما حثت تلك المنظمات على انشاء واعتماد معامل ومراكز دولية فى مجال الكشف عن المنشطات باستخدام بعض الطرق الكيميائية المختلفة.

نظرا لاهمية هذا الموضوع ودورنا لتطوير واكتشاف طرق جديده للكشف عن المنشطات تم تحضير وتوصيف احد المتراكبات الجديده وهو متراكب التربيوم -الاسيتل اسيتات , كما تم اجراء بعض التطبيقات باستخدام هذه المتراكب بغرض كشف وتقديراحد هذه العقاقير المنشطة وهو مركب الهيدروكلوروسيازيد في صورته الطبيعية أو فى السوائل البيولوجية وتطبيق هذه التقنية لتقدير هذا المركب في بعض تركيباتها الصيدلية وذلك باستخدام طرق التقدير الوميضى.

وقد تم استخدام بعض الأجهزه في التعرف على تركيب المتراكب الناتج وخواصه التحليليه والطيفيه وتوصيفها , ثم توصيف طريقة وميضية مباشرة لتقديرمركب الهيدروكلوروسيازيد اعتمادا على قياس الطيف الوميضى عند اس هيدروجيني مناسب. تم الحصول على علاقة خطية بين شدة الطيف الوميضى وتركيز هذا المركب. ولقد أظهرت النتائج دقة عالية ولقد تم تطبيق الطريقة بنجاح .