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Spectrophotometric Determination of Thioridazine Hydrochloride in Tablets and Biological Fluids by Ion-Pair and Oxidation Reactions

Akram El-Didamony[1] and Sameh Hafeez[2]

[1] *Chemistry Department, Faculty of Science, Zagazig University, Zagazig 44511, Egypt*
[2] *Ismailia Chemical Laboratory, Forensic Medicine Authority, Justice Ministry, Ismailia, Egypt*

Correspondence should be addressed to Akram El-Didamony, ak_eldidamony@yahoo.com

Abstract. Two simple, sensitive and selective spectrophotometric methods have been described for the determination of the psychoactive drug, thioridazine HCl in tablets and in biological fluids. The first method is based on the oxidation of thioridazine HCl with measured excess of $KMnO_4$ under acidic conditions followed by the determination of unreacted oxidant using indigo carmine and methyl orange. The second method is based on the formation of ion-pair complexes with the acidic sulphophthalein dyes such as bromocresol green and bromocresol purple at pH 1.8 of KCl-HCl buffer. The formed complexes were extracted into methylene chloride and their absorbance was measured at 412 nm. Optimizations of the different experimental conditions are described for both methods. The proposed methods were successfully applied for determination of the drug in tablets and biological fluids with good accuracy and precision. Statistical comparison of the results with those obtained by an official method showed good agreement and indicated no significant difference in accuracy and precision.

Keywords: Spectrophotometry, thioridazine HCl, $KMnO_4$, indigo carmine, methyl orange, sulphophthalein dyes

1. Introduction

Thioridazine hydrochloride (THZ), the hydrochloride of 10-[2-(lmethyl-2-piper-idyl)ethyl]-2-methylthiophenothiazine, is a phenothiazine neuroleptic drug used for the treatment of schizophrenia and other psychiatric disorders [1]. Phenothiazines also possess antiemetic, sedative, antipruritic, antidyskinetic, analgesic, and antihistaminic properties [2]. Thioridazine has been known as one of the most potent phenothiazines which inhibit trypanothione reductase irreversibly and is commonly used as an antipsychotic and antimuscarinic drug to treat behavioral symptoms [3, 4]. Additionally, thioridazine was effective in the treatment of sweating as an antidepressant and sedative drug [5, 6].

Due to the clinical importance of thioridazine hydrochloride, it is significant to establish a simple and sensitive method for its determination in biological fluids. Different pharmacopoeias recommend

the determination of thioridazine base or the hydrochloride by titrating the drug in glacial acetic acid and acetic anhydride against perchloric acid. The BP recommends the titration of the drug in acetone solution containing about 7% mercury(II) acetate solution and using methyl orange as indicator [7]. Several analytical methods have been described for determination of thioridazine hydrochloride. Among the methods, UV spectrophotometry and conventional high performance liquid chromatography are most often used [8–11]. Voltammetry [12–14], spectrophotometry [15–19], and chemiluminescence methods have been reported for the determination of thioridazine hydrochloride [20]. Supercritical fluid chromatography [21] and LC combined with other techniques were also reported for determination of thioridazine hydrochloride [22, 23] and their main metabolites or derivatives. Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop two new simple and sensitive methods for the spectrophotometric determination of THZ. The first method is based on the oxidation of THZ with slight excess of potassium permanganate in acidic medium. The unconsumed of oxidant is then estimated with the decrease in the color intensity of two dyes. The second method is based on the formation of ion-pair complexes of drug with dyestuffs such as bromocresol green (BCG) and bromocresol purple (BCP) and subsequent extraction into methylene chloride. The proposed methods have been successfully applied for the determination of THZ in pharmaceutical formulations and biological fluids.

2. Experimental Section

2.1. Instruments

A spectroscan 80 D double-beam UV/visible spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190–1100 nm, spectral bandwidth 2.0 nm with matched 10 mm quartz cells were used for all the absorbance spectral measurements.

2.2. Reagents and Materials

All chemicals used were of an analytical reagent grade and solutions were made in distilled water.

(i) Indigo carmine (IC). A 5×10^{-3} M of indigo carmine (Aldrich) was prepared by dissolving of an accurate weight dye (99% purity) in water and diluting to 100 mL in a calibrated flask.

(ii) Methyl orange (MO). A 5×10^{-4} M of methyl orange was prepared by dissolving an accurate weight of dye in least amount of water and completed to the mark in a 100 mL calibrated flask.

(iii) Potassium permanganate. A stock solution of 1.0×10^{-3} M $KMnO_4$ (Aldrich) was prepared by dissolving an accurate weight in 10 mL of warm distilled water, then completed to the mark in a 100 mL calibrated flask, and standardized using sodium oxalate. A 2.0 M H_2SO_4 was prepared.

(iv) Sulfophthalein dyes. Solutions of 5×10^{-4} M bromocresol green and bromocresol purple (Aldrich product) were prepared by dissolving an accurately weight of the acid dyes in a few drops of acetone and then diluted to the mark with distilled water in a 100 mL calibrated flasks separately.

(v) Thioridazine hydrochloride (THZ). Pharmaceutical grade thioridazine hydrochloride was received as a gift from Delta Pharm Company, 10th of Ramadan, Egypt; it was reported to be 99.8% pure and was used as received. A stock standard solution equivalent to 10 mg of THZ was prepared by dissolving an accurately weighed amount of pure drug in distilled water. The solution of THZ was diluted stepwise to obtain working concentration of 100 μg/mL. The standard solutions were kept in amber-colored bottles and stored in a refrigerator when not in use.

2.3. Recommended Procedures and Calibration Curves

2.3.1. Oxidation Reaction

Varying aliquots (0.1–0.7 mL) of the standard 100 μg/mL THZ solution were transferred into a series of 25 mL calibrated flasks by means of a microburette. To each flask, 2.0 mL of 2.0 M H_2SO_4 and 1.2 mL of 1.0×10^{-3} M $KMnO_4$ solution were added. The content was mixed well and the flasks were kept aside for 20 min, at room temperature with intermittent shaking. Finally, 1.1 mL of 5×10^{-3} M of IC and 1.4 mL of 5.0×10^{-4} M of MO solution were added to each flask and the volume was diluted to the mark with water and mixed well. The absorbance was measured against a reagent blank at 610 nm and 508 nm, respectively. In both methods, a standard graph was prepared by plotting the absorbance versus the concentration of THZ. The unknown concentration was read from the calibration graph or computed from the regression equation derived using Beer's law data.

2.3.2. Ion-Pair Reaction

Suitable aliquots of the stock solution of THZ (4–24 μg) for both BCG and BCP methods were transferred into separate glass-stopper tubes. Then, 2.0 mL KCl + HCl buffer of pH 1.8 and 2.0 mL of 5×10^{-4} M BCG or BCP were added and mixed. The contents were extracted with 10 mL methylene chloride by shaking for 2.0 min and the organic layer was dried over anhydrous sodium sulphate. The absorbance of the yellow-colored complexes was measured at 412 nm for both BCG and BCP, against a methylene chloride. In both methods, a standard graph was prepared by plotting the absorbance versus the concentration of THZ.

2.4. Procedure for Pharmaceutical Preparations

At least twenty of THZ tablets (Thiozine tablets, Delta Pharm Company, Cairo, Egypt, are labeled to contain 25 mg of thioridazine HCl per tablet) were weighed to obtain the mean tablet weight and then ground to a homogenized powder. A quantity of the powdered tablets equivalent to 30 mg was transferred into a 100 mL calibrated flask and dissolved in distilled water and filtered. A suitable amount of filtrate was taken and analyzed as described under recommended procedures. For the proposed methods, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

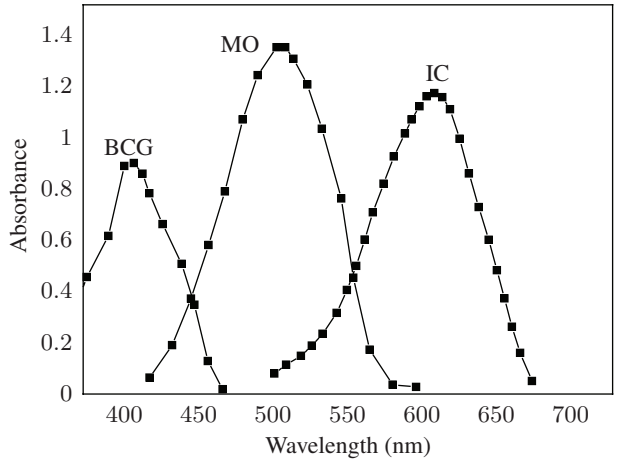


Figure 1: Absorption spectra of the reaction products.

2.5. Procedure for Spiked Urine and Serum

The proposed methods were applied to the determination of THZ in spiked urine and serum provided from several healthy volunteers. Spiked urine was 50-fold diluted with distilled water. A 10 mL of serum sample was deproteinized by adding 5 mL of acetonitrile in a centrifuge for 5 min at 1000 rpm. The supernatant was used to investigate recovery. Add an aliquot of standard aqueous solution of THZ to 1.0 mL of diluted urine or serum. The analysis was completed as in the recommended procedures. A blank value was determined by treating THZ-free urine and THZ-free serum in the same way. The absolute recovery was determined for THZ by comparing the representative absorbance of the treated urine or serum samples with the absorbance of the standard drug at the same concentration.

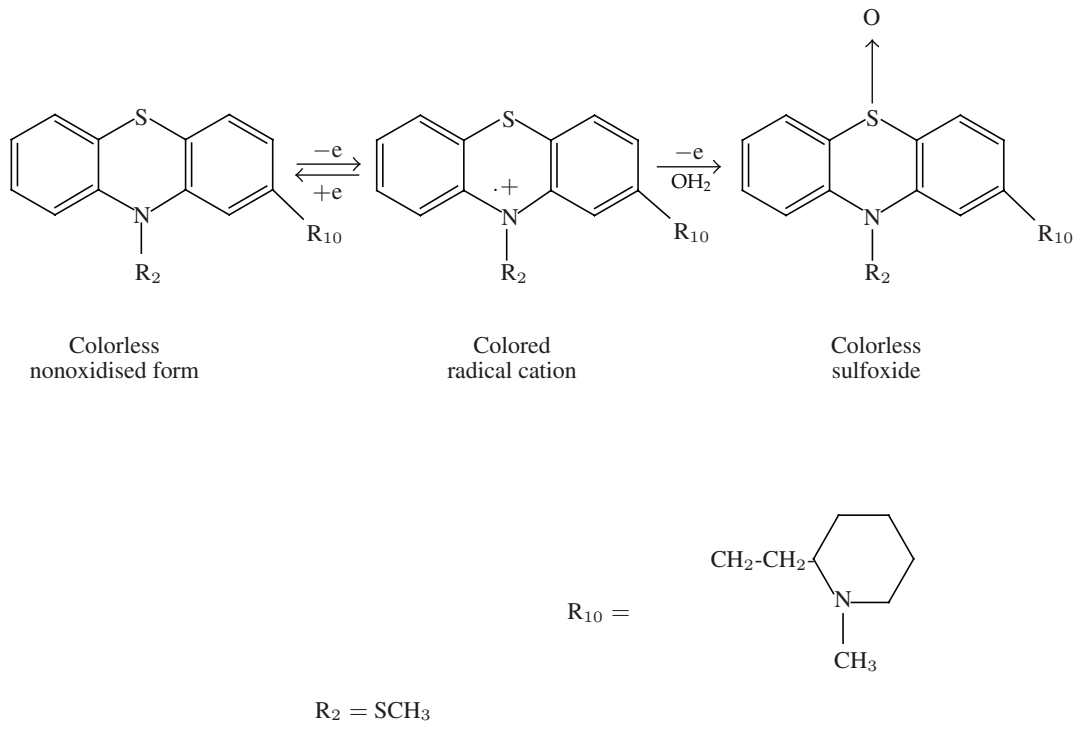
3. Results and Discussion

Optimization of Variables

The optimum conditions for the assay procedures have been established by studying the reactions as a function of reaction time, concentration of reagents, and stability of the colored species, buffer and pH. Such variables were changed individually while the others were kept constant.

3.1. Oxidation Reaction

In the present method, two dyes indigo carmine and methyl orange have been used for the determination of THZ (Figure 1). The determinations of THZ are indirect and are based on the determination of surplus $KMnO_4$ after the oxidation reaction of THZ by $KMnO_4$, [24, 25]. The drug undergoes oxidation according to the reaction scheme given in Scheme 1.



Scheme 1: The probable mechanism for the oxidation of THZ.

3.1.1. Effects of Temperature and Reaction Time

Keeping other conditions constant, the effect of temperature on the oxidation product was studied. The reaction between THZ and $KMnO_4$ was found to be instantaneous. However, the reaction is complete within 15 min at room temperature ($25 \pm 2°C$), but 20 min was sufficient to get maximum intensity. Therefore, 20 min at room temperature have been selected for further experiments.

3.1.2. Effect of Acid Concentration

Preliminary investigation showed that sulphuric acid was the medium of choice for the oxidation of THZ by $KMnO_4$. Two milliliters of 2.0 M H_2SO_4 were ideal for the oxidation step in both methods and the same quantity of acid was employed for the estimation of the dye. It was found that the maximum color was developed within 20 min and remained almost stable for about 24 h.

3.1.3. Effect of Oxidant Concentration

The effect of oxidant concentration was studied by adding different volumes of 1×10^{-3} M of $KMnO_4$ solution to a constant amount of THZ (2.4 μg/mL). It was observed that the maximum color intensity was obtained with 1.2 mL of $KMnO_4$, after which further increase in volume resulted in a decrease of

absorbance. Thus, 1.2 mL of $KMnO_4$ was sufficient to reach with the maximum drug concentration in the Beer's range.

3.1.4. Effect of Dye Concentration

The effect of the dyeconcentration on the intensity of the color developed at the selected wavelengths was studied separately by measuring the absorbance's of final solutions resulting from reaction mixtures containing a fixed concentration of THZ (2.4 μg/mL) and various amounts of dyes (0.5–5 mL). The use of 1.1 mL of 5×10^{-3} M IC and 2.8 mL 5×10^{-4} M of MO was found to be necessary to produce constant absorbance values. The use of excess of reagent produced no further increase in absorbance.

3.2. Ion-Pair Reaction

Extractive spectrophotometric procedures due to their sensitivity are widely used in the assay of drugs and, hence, ion-pair extractive spectrophotometry has received a considerable attention for the quantitative determination of many pharmaceutical compounds [26–31]. THZ reacts with BCG and BCP in an acidic buffer to give methylene chloride soluble ion-pair complexes, which exhibit absorption maxima at 412 nm for BCG and BCP, (Figure 1). Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for the quantitative determination of THZ.

3.2.1. Effect of Buffer and pH

Optimum reaction conditions for the quantitative determination of ion-pair complexes were established via a number of preliminary experiments. It was observed that the effective extraction of the complex depended on the type of buffer used and its pH. The effect of pH was studied by extracting the colored complexes in presence of various buffers namely, KCl-HCl (pH = 1.0–2.2), potassium hydrogen phthalate-HCl (pH = 2.2–3.6), NaOAc-HCl (pH = 1.99–4.92), and NaOAc-AcOH (pH = 3.72–5.57). It was noticed that the maximum color intensity and constant absorbances were observed in KCl-HCl buffer (Clark and Lubs) of pH 1.8 for both dyes. Hence, Clark and Lubs buffer of pH 1.8 was used for all subsequent measurements in both methods. Further, 2.0 mL of KCl-HCl buffer of pH 1.8 for both BCG and BCP gave maximum absorbances and reproducible results.

3.2.2. Choice of Organic Solvent

A number of organic solvents such as chloroform, dichloromethane, carbon tetrachloride, benzene, and toluene were examined for extraction of the ion-pair complexes in order to provide an applicable extraction procedure. Although dichloromethane is not an ecologically friendly solvent, it was preferred for its selective extraction for ion-pair complexes due to the greater stability of the extracted ion pair and considerably lower extraction abilities of the reagent blank. The study reveals that a volume ratio of 1 : 1 (aqueous : organic) was the most suitable for ion-pair extraction.

3.2.3. Effect of Time and Temperature

The effect of temperature on the colored ion associates was studied at different temperatures. It was found that the colored ion associates were stable up to 30°C. Higher temperatures were not suitable due to the volatile nature of methylene chloride. The resulting ion associates were found to be stable up to 24 h at room temperature. Complete color intensity was attained after 2.0 min of mixing for all complexes.

3.2.4. Effect of Dyes Concentration

The effect of dyes' concentrations were studied separately by measuring the absorbance's of final solutions resulting from reaction mixtures containing a fixed concentration of THZ and various amounts of the dyestuff. Maximum color intensity of the complex was achieved with 2.0 mL of 5×10^{-4} M of BCG or BCP. Although a larger volume of the reagent had no pronounced effect on the complex formation, the absorbance's increased slightly due to background of the colored reagent.

3.2.5. Effect of Sequence and Time of Shaking

The most favorable sequence was drug-buffer-dye-solvent for the production of the highest color intensity and the shortest time for developing maximum absorbance, while the other sequences require longer time and produce lower absorbance values. The time of shaking for extraction of ion-pair complexes was studied and found that the absorbance of the extract remains constant between 0.5–5.0 min. Thus, 2.0 min shaking time was utilized as an optimum value throughout the experiment. The ion-pair complexes were quantitatively recovered in one extraction only.

3.2.6. Stoichiometric Relationship

The stoichiometric ratio of the formed products was investigated by Job's continuous variation method [31]. A 1×10^{-4} M solution of THZ was used with comparable solutions of the reagent (BCG or BCP). For each method, a series of solutions was prepared in which the total volume of the drug and reagents was kept at 2.0 mL, and the procedure was completed as described under general procedures. The application of Job's continuous variation method indicated 1 : 1 ratio for the drug with each reagent.

3.3. Analytical Data

The calibration graphs were constructed for the two methods under the optimum conditions described above. The molar absorptivity, concentration range, regression equation, and correlation coefficient are tabulated in Table 1. A linear relationship was found between the absorbance at $\lambda_{\max}$ and the concentration of the drug in the range 0.4–3.2 μg/mL for oxidation method and 4–32 μg/mL for ion-pair method. Regression analysis of the Beer's law plotted at $\lambda_{\max}$ reveals a good correlation (r^2 = 0.9972–0.9993). The graphs showed a negligible intercept, which was calculated by the least squares

Table 1: Optical characteristics and statistical data for the regression equation of the proposed methods.

Parameters	Ion-pair		THZ + $KMnO_4$	
	BCG	BCP	IC	MO
λ_{max} (nm)	412	412	610	508
Beer's law limit (μg/mL)	4–28	4–32	0.4–3.2	0.4–2.4
Molar absorptivity (L/mol cm)	1.78×10^3	1.58×10^3	3.82×10^4	5.28×10^4
Sandell's sensitivity (μg/cm^2)	0.2293	0.2568	0.0106	7.71×10^{-3}
Correlation coefficient (r)	0.9988	0.9972	0.9980	0.9993
Linear regression equation[a]				
$S_{y/x}$	0.01516	0.02157	0.0105	0.0104
Slope (b)	0.0422	0.0392	0.24	0.2455
Intercept (a)	0.014	0.1087	0.4912	0.635
S.D. of slope (S_b)	1.19×10^{-3}	1.71×10^{-3}	8.29×10^{-3}	8.25×10^{-3}
S.D. of intercept (S_a)	6.43×10^{-3}	9.15×10^{-3}	0.0223	0.0222
LOD (μg/mL)	—	—	0.0583	0.0641
LOQ (μg/mL)	—	—	0.1943	0.2133

[a] $A = a + bC$, where A is the absorbance and C is the concentration of THZ in μg/mL.

method's regression equation. The relative sensitivities of the reagents can be determined by comparing the molar absorptivities of the complexes.

3.4. Accuracy and Precision

The interday repeatability of the proposed methods was studied by performing five independent analyses of THZ in pure form at three different concentration levels on six consecutive days (Table 2). The intraday reproducibility of the proposed method was determined by measuring the drug at three concentration levels within one day five times (Table 2). The reagent solutions were prepared freshly and analyzed as described under recommended procedures and calibration graphs. Data of Table 2 show that within day the relative standard deviations are less than 1.8% and interday relative standard deviations are not exceeding 2.17%, which indicates that the proposed methods are highly reproducible.

3.5. Analytical Applications

3.5.1. Analysis of Tablets

The proposed methods were successfully applied to the determination of THZ in representative tablets and the results are summarized in Table 3. For the brands/doses examined, the methods gave results which were in agreement with the declared content. The performance of the proposed methods was judged by calculating the student t- and F-values. At 95% confidence level, the calculated t- and F-values did not exceed the theoretical values as evident from Table 3. Hence, it was concluded that there

Table 2: Evaluation of intraday and interday accuracy and precision of the proposed methods.

Method	THZ taken μg/mL	Intraday			Interday		
		THZ found[a], μg/mL	RE[b], %	RSD, %	THZ found, μg/mL	RE, %	RSD, %
BCG	8	7.999	−0.002	1.653	7.999	−0.002	2.025
	16	15.999	−0.004	0.796	15.999	−0.004	1.259
	24	23.999	−0.002	0.421	23.999	−0.002	1.165
BCP	8	7.9996	−0.004	0.853	7.9996	−0.002	2.174
	16	15.999	−0.006	1.312	15.999	−0.006	1.774
	24	23.999	−0.002	0.728	23.999	−0.002	1.927
$KMnO_4$ + IC	0.8	0.801	0.026	1.236	0.799	-4×10^{-3}	0.929
	1.6	1.599	-4×10^{-3}	1.052	1.599	-2×10^{-3}	0.974
	2.4	2.399	-2×10^{-3}	0.717	2.399	-2×10^{-3}	0.424
$KMnO_4$ + MO	0.4	0.399	-4×10^{-3}	1.648	3.999	-4×10^{-3}	1.355
	0.8	0.799	-2×10^{-3}	1.807	0.799	-2×10^{-3}	1.889
	1.6	1.599	-2×10^{-3}	1.660	1.599	-4×10^{-3}	0.803

[a] Mean value of five determinations.
[b]RE: relative error.

Table 3: Recovery of THZ in tablets formulation using the proposed methods.

Formulation	Found[a] (% of nominal amount ± RSD)				
	THZ-BCG	THZ-BCP	$KMnO_4$ + IC	$KMnO_4$ + MO	Official method
Thiozine 25 mg/tablet	98.69 ± 1.66	97.93 ± 1.72	98.92 ± 1.51	99.92 ± 0.78	99.88 ± 0.901
	$t = 2.064$	$t = 1.487$	$t = 2.046$	$t = 1.876$	
	$F = 3.394$	$F = 3.644$	$F = 2.808$	$F = 1.334$	

[a] Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.77.
Tabulated F-value at the 95% confidence level is 6.39.

is no significant difference between the proposed and official methods [7] with respect to accuracy and precision. Moreover, to check the validity of the proposed methods, we applied the standard addition method by adding THZ to the previously analyzed tablets. The recovery of THZ was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug and the recoveries of added quantity were found to be more than 96.29%. This indicates that there is no interference from any excipients, which are present in tablets. These results are given in Table 4.

Table 4: Results of the recovery study by the standard addition method.

Formulation	Method	Amount of drug in formulation, μg	Amount of pure drug added, μg	Total found, μg	Recovery %	RSD, %
Thiozine, 25 mg/tablet	BCG	8	12	11.661	97.17	1.2601
			16	15.745	98.41	1.1922
			20	19.258	96.29	1.8082
	BCP	8	12	11.725	97.71	1.4406
			16	15.812	98.82	1.2663
			20	19.587	97.94	1.9331
	$KMnO_4$ + IC	1.2	0.4	1.610	100.68	0.9703
			0.8	1.974	98.74	1.6282
			1.0	2.174	98.81	1.4965
	$KMnO_4$ + MO	0.8	0.4	1.189	99.16	1.1380
			0.8	1.581	98.85	1.4635
			1.2	1.974	98.77	1.5673

3.5.2. Analysis of Human Samples

Phenothiazines are used as antipsychotic drugs and as reagents to determine the Hb in biological fluids and tissues, but over dosage can cause death, so the quantitative assessment of added thioridazine in serum and urine of healthy human beings is also determined as shown in Table 5. High accuracy and good recovery are obtained, which indicate that the proposed methods can be successfully applied to recover thioridazine in human samples.

3.6. Interference Study

The commonly used excipients and additives which often accompany THZ in its dosage forms, such as (starch, lactose, glucose, sugar, talc, sodium chloride, and magnesium stearate) was studied. The results indicated that there is no interference from the degradation, indicating a high selectivity for determining the studied THZ in its dosage forms.

3.7. Robustness

The robustness of the proposed method was evaluated by using the different instruments by two different analysts under the same optimized conditions. The obtained results were found to be reproducible, since there was no significant difference between the results obtained by the two analysts. Thus, the proposed methods could be considered robust.

Table 5: Recovery of THZ in serum and urine ($n = 5$, $t = 2.78$).

Method	Added, μg/mL	Serum			Urine		
		Found, μg/mL	Recovery, %	RSD, %	Found, μg/mL	Recovery, %	RSD, %
BCG	8	8.0605	100.75	3.8828	7.9145	98.93	2.8539
	16	15.9593	99.74	1.7306	15.9395	99.62	1.2147
	24	23.9661	99.85	0.8900	24.0289	100.12	0.8345
BCP	8	8.1462	101.82	2.4969	7.9788	99.73	1.9612
	16	16.0048	100.03	0.8643	15.9067	99.41	0.9044
	24	23.9469	99.77	0.7588	23.7888	99.12	1.2083
$KMnO_4$ + IC	0.4	0.3999	99.996	1.633	0.80	100.00	1.839
	0.8	0.7999	99.996	2.418	1.1751	97.93	2.612
	1.6	1.5999	99.996	1.779	1.5645	97.78	2.849
$KMnO_4$ + MO	0.8	0.7999	99.998	0.8563	0.4059	101.49	2.109
	1.2	1.1999	99.996	1.927	0.80	100.00	1.025
	1.6	1.5999	99.996	0.846	1.1999	99.99	0.7039

4. Conclusions

The data given above reveal that the proposed methods are simple, selective, accurate, and sensitive (oxidation method $>$ ion-pair method) with good preciseness and accuracy. Thus, the proposed methods can be used as alternative methods to reported ones for the routine determination of THZ in pharmaceutical preparations and in biological fluids depending upon the availability of chemicals and the nature of other ingredients present in the sample.

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