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High throughput flow injection /MZ technique for indirect assay of hydrosulfurnyl group in Tiopronin drugs and biological samples using 2,2`-dipyridyl as a selection OAR

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Abstract

A simple, automated and sensitive continuous flow-injection analysis merging zones technique (CFIA/MZ) method was developed to determine Tiopronin in pure, pharmaceutical formulations and biological samples. This method included a reducing reaction between Tiopronin with Fe (III) to produce Fe (II) and then the Fe (II) react with 2,2`-dipyridyl to produce a Pink-red color complex which has maximum absorbance at the wave length 522nm. FIA/MZ was cheap, economical, accurate and precise which the detection limit was 4.0670 µg.mL^{-1} and RSD% percent about 0.7925% and the recovery is 101.93. The reaction was studied under a number of chemical and physical parameters. concentrations ranging from 5 to 200 µg.mL^{-1}, the calibration curve was rectilinear with a sample throughput of 63 sample.hour^{-1}. The proposed method was applied successfully to the estimation of Tiopronin in pharmaceutics and biological samples and the acquired results were compared well to those produced by a Chinese Pharmacopoeia standard technique, and there was no a significant difference between the obtained results in terms of accuracy and precision at the 95 percent confidence level.

Keywords

Keywords: Tiopronin, CFIA/Spectrophotometric system, Modified detection unit, Pharmaceutics, 2,2`-dipyridyl;

Introduction

Tiopronin (TIO) ($C_5H_9NO_3S$), M.wt=163.19 g.mol^{-1}, the IUPAC name is (2-Sulfanylpropanamido)acetic acid, Figure 1 shows the chemical structure of TIO, TIO is a glycine derivative containing reductive sulfhydryl group and widely used in the clinical treatment of liver diseases. TIO is officially in Chinese Pharmacopoeia [1], which is based on titration method for the detection of TIO in raw materials or pharmaceutical formulations. There are many analytical techniques can be determination of TIO in the dosage form of pharmaceutical including Amperometric Flow Injection [2], Chemiluminescence [3,4], Fluorescence [5-9], HPLC [10-12], LC–ESI–MS [13], Liquid Chromatography [14], Spectrophotometric [15-18], Capillary Electrophoresis [19] and Voltammetric [20]. These methods described that are time consuming and require multistage extraction procedures, although the procedures are specific. Reported spectrophotometric methods result from one or the other disadvantage such as poor sensitivity, use of organic solvent, the problems of extraction, scrupulous control of experimental variables and special equipment, or small linear ranges. The proposed method of Flow Injection analysis (CFIA/MZ) in the manuscript was used for indirect determination of TIO using [Fe (III) - TIO - 2,2`-dipyridyl] system [21-24].

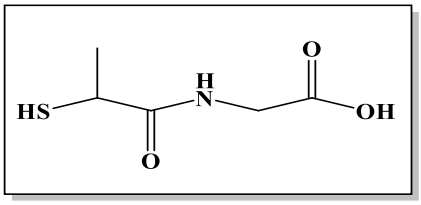


Figure 1: formula structure of Tiopronin.

Apparatus and FIA Manifold

On optima VIS 9200, digital single beam applied all of spectral absorbance placed in the sensor quantifications on its by recording the spectrophotometer with flow cell (quartz silica, 1cm) 80 µL internal volume is inside the detection unit and 1cm an optical path length using for the absorbance measurements as average peak height expressed in mV (n=3). A one-channel manifold with Flow Injection Analysis-merging zones for spectrophotometer TIO estimation is working in this paper. A power supply (Yaxun, 1501AD, China) was used a Peristaltic pump (Master flexC/L, two channel, USA) to pump a carrier stream in injection (distilled water) and solutions through a home-made injection valve (seven-three-way injection valve with three loops). chemicals and

reagents solutions which are based on merging zones version [21,22]. The injection valve that used to supply suitable volumes that were injected with samples and standard solutions. For the peristaltic pump, flexible vinyl tubes with radius of 0.22 mm were used; the mixing and reaction coil was constructed of glass with a diameter of 2 mm (I.D). All of the parts of the CIFA as shown in Figure 2 with details. Distilled water served as a carrier stream that was mixed with Tiopronin (TIO) in L1, $FeCl_3$ in L2 and 2,2`-dipyridyl (BPY) as a reagent in L3. Then mixed all together in a mixing coil that has length of 50 cm and the carrier flow rate is 8.1 $mL.min^{-1}$. The maximum absorption was found at 522 nm Pink-red color complex as peak height in (mV).

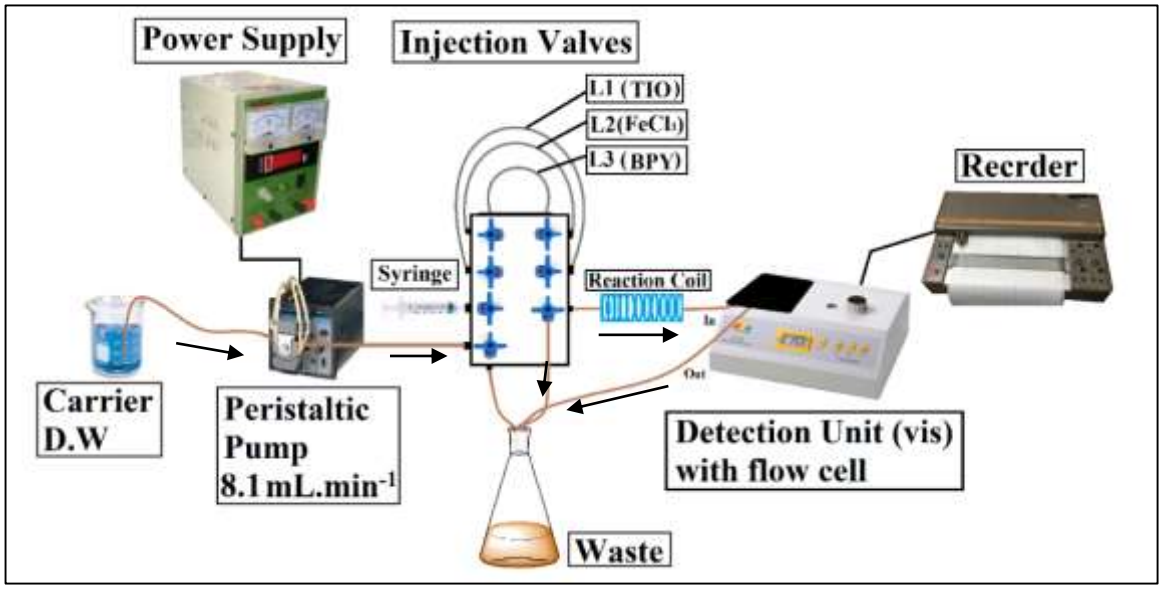


Figure 2: The Manifold CFIA system for determination of TIO drug.

Reagents and Chemicals

All of the chemicals and reagents utilized were analytical grade, and all of the solutions were prepared with them.

- **Tiopronin (TIO) stock solution** (M.wt=163.19 $g.mol^{-1}$, Merck, Germany): (1000 $\mu g.mL^{-1}$ = 6.1×10^{-3}M): A TIO (100 mg) was dissolved in distilled water then be consummated to 100 mL in standard flask with distilled water. The diluted solutions prepared by adequate diluting of the stock standard solution with distilled water.
- **2,2`-dipyridyl (BPY) solution** (M.wt = 156.19 $g.mol^{-1}$, Merck) (1.3×10^{-3} M) : A BPY (0.05 g) was dissolved in ethanol then the volume was made to 250mL in standard flask and farther dilution to these solutions to obtain desired concentrations.
- **Ferric Chloride ($FeCl_3$) solution** (M.wt= 162.20 $g.mol^{-1}$) (1.2×10^{-3} M) : A $FeCl_3$ (0.05 g) was dissolved in distilled water then the volume was made to 250 mL in standard flask and farther dilution to these solutions to obtain desired concentrations.

Preparations of TIO Pharmaceutical (1000 µg. mL^{-1})

The trading sources for gained pharmaceutical formulation obtainable tablet and powder from fore kinds companies were assayed by the procedure proposing. the various companies for different providers were including:

1. Thiola tablet 100mg, USA
2. Tiopronin teva tablet 100 mg, USA
3. Tiopronin Care tablet 100 mg, India
4. Deutsch-Tiopronin 100 mg, China

Further solutions were diluted to prepare the concentration inside of the linearity of the calibration graph. Recovery experiment was performed by applying the standard-addition technique [25]. The agreement between the measured concentration and the final known concentration to the sample was used to assess the recovery. Each test was repeated three times in total.

Preparation of biological samples

- **Serum samples** with gently thawing, the sample was obtained from a healthy volunteer and stored at 20°C until use. For serum sample preparation 100 $\mu g.mL^{-1}$ was tested for accuracy and precision and analyzed thrice [24].
- **Urine samples preparation** the sample was collected from a healthy volunteer and stored at 20 °C until use after gentle thawing. For urine sample preparation 100 $\mu g.mL^{-1}$ of TIO [24].

Result and discussion

Batch method

Increasing volumes (0.5-4) mL of 100 µg.mL^{-1} TIO were added into a set of 10 mL standard flask then 2 mL of (1.2 × 10^{-3} M) $FeCl_3$ was transferred and 0.5 mL of 1.3 × 10^{-3} M BPY was added and consummate the volume of the solutions to the mark with distilled water. The maximum absorption of the Pink-red color complex was found at λ_{max} 522 nm opposite the blank solution.

Absorption spectra

The last concentration of 10 µg.mL^{-1} TIO was reacted with 6.5 × 10^{-5} M of $FeCl_3$ and 2.4 × 10^{-4} M BPY to give the colored product complex which was examined under visible spectrum (from 350-650) in order to determine the maximum absorbance for the complex and it was clear that the λ_{max} was 522 nm for the Pink-red color complex as shown in Figure 3.

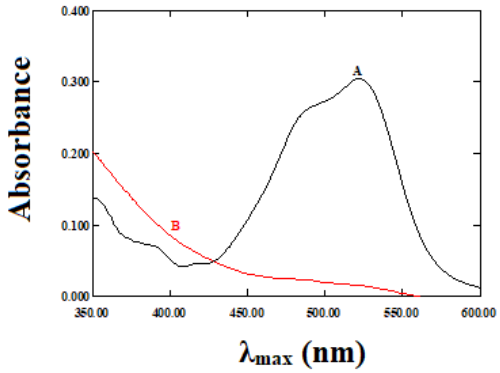
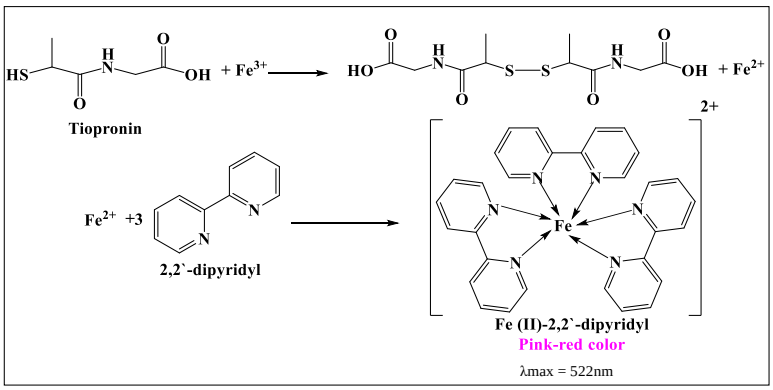


Figure 3: The Absorption spectrum of: A\ Pink-red color complex against blank solution (BPY and $FeCl_3$), B\ blank solution against distilled water.

The proposed mechanism of the reaction

The spectrophotometric determination of TIO was based on reduction of Fe (III) in the presence of TIO as reducing agent to form Fe (II), then Fe (II) react with BPY to produce a Pink-red color complex (Scheme 1) [18].



Scheme 1: Reduction of Fe (III) by TIO and formation of a Pink-red color complex between Fe (II) and BPY.

The ratio of reactions that happened through the reagent and the drug by two ways was preceded by mole ratio and continuous variation techniques (Job's method) and 1:3 ratio was for reagent and the drug as shown in Figure 4.

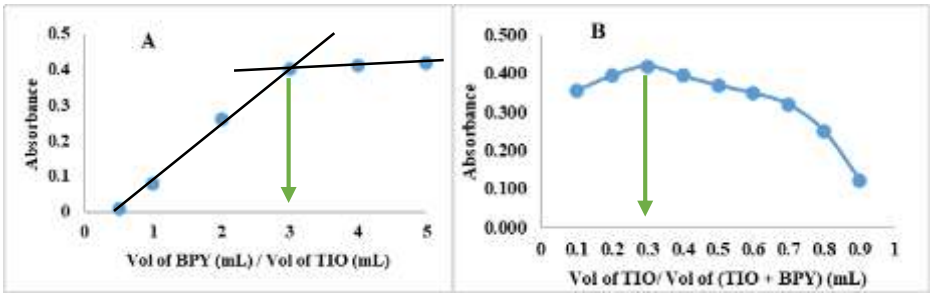


Figure 4: The complexation ratio between a reagent with drug, A\ mole ratio for the complex, B\ job's method for the complex.

Preliminary investigation

The effect of BPY volume was examined with 10 µg.mL^{-1} TIO. It has been monitored that the volume that gives the highest absorbance was 0.5 mL of 1.3 × 10^{-3} M BPY and this volume was selected for subsequent experiences, as shown in Figure 5-A. The oxidative agent $FeCl_3$ examined with TIO and the best volume was 2 mL of 1.2×10^{-3} M $FeCl_3$ as shown in Figure 5-B.

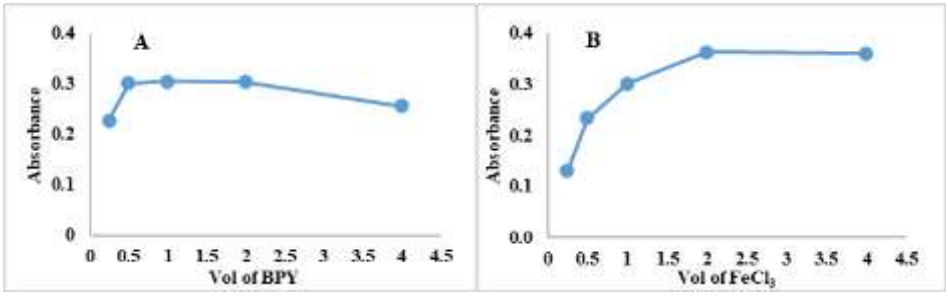


Figure 5: Chemical parameter for batch A/ volume of BPY, B/volume of $FeCl_3$.

Calibration curve of classical method

The standard curve was constructed with a linear range (5-40) µg.mL^{-1} for the estimation of TIO, as shown in Figure 6.

Precision and Accuracy

based on the ideal conditions explained in established method, these measuring were by two different levels of TIO for precision and accuracy, these results, which showed in Table 1, that the suggested method does have good precision and accuracy, and these measurements were done five times.

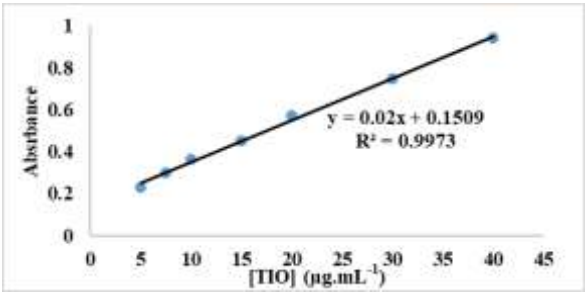


Figure 6: Linear calibration curve for determination of TIO drug using Batch method.

Table 1: Precision and accuracy.

TIO (µg. mL^{-1})		E	Rec%	Erel %	RSD %
Present µ	*Found $\bar{x}$				
10	10.355	0.355	103.55	3.550	0.4417
20	20.455	0.455	102.28	2.275	0.2823

Average of five determinations

Calculations of stability constant

An observed stability constant [26,27] for the proposed interaction (TIO: BPY) was determined using two groups of solutions: first one does include a stoichiometric amount of TIO to BPY, while the second includes a two-fold excess of BPY. According to the suggested mechanism and drug-to-reagent stoichiometry ratio (1:3). The reaction between TIO and BPY proceeds according to the equation:

$$\underset{\alpha C}{D} + \underset{3\alpha C}{3R} \rightleftharpoons \underset{(1-\alpha)C}{DR_3} \qquad K = \frac{[DR_3]}{[D]\,[R]^3} \qquad K = \frac{(1-\alpha)}{4\alpha^4 C^3} \qquad \alpha = \frac{Am - As}{Am}$$

While K is the stability constant, C is the product's molar concentration (M), which is the same as the concentration of TIO (1×10^{-4} M), (α) is the degree of dissociation. Where Am; As are the absorbance values of the aqueous solution, which includes a sufficient and stoichiometric quantity of reagent. The spontaneous of complex formation reaction (ΔG value) was estimated based on K evaluation as in Table 2 and the equation: ΔG = -RT lnK

where ΔG: Gibbs free energy, R: general constant of gases (8.314 J. mol^{-1}. K^{-1}), T: absolute temperature (298.15 K).

Table 2: stability constants and Gibbs free energy of the reaction.

	Am	As	α	K (L^3.mol^{-3})	ΔG (J.mol^{-1})
1	0.443	0.403	0.09029	5.1 × 10^{14}	-83931
2	0.440	0.405	0.07955	8.5 × 10^{14}	-85217
Average				6.8 × 10^{14}	-84574

Flow injection/ Merging zones spectrophotometric determination

After selecting the optimum conditions of reaction of TIO with BPY in the classical spectrophotometric method. The spectrophotometric reaction was automated with flow injection-merging zones technique to study the best practical parameters and to obtain spectra automated with a fast way to estimate TIO. So, the batch procedure for estimation of TIO was employed as a basis to develop flow injection analysis methods.

Manifold of FIA system

With the installation of the system and its connected components, the investigation of the best design of a Homemade FIA system began. The developed system shown in Figure 2 is composed of one line supplies the carrier is distilled water leading to the injection valve; which contain three loops (different loop length with 0.5mm I.D.) that fills by the drug, reagents and oxidized agent according to the order TIO in L1, $FeCl_3$ in L2 and BPY in L3.

Optimization of the developed FIA system conditions

Chemical variables

The optimal concentration of the reagent BPY was investigated by injecting various concentrations ($6.5 \times 10^{-5} – 1 \times 10^{-3}$) M using a handmade injection valve loading in loop (L2). The concentration 2.6×10^{-4} M produced the best value of absorbance expressed as peak height in mV (n = 3) and high repeatability, which is shown in Figure 7-A. The best concentration of the oxidized agent $FeCl_3$ was investigated by injecting several concentrations ($6 \times 10^{-5} – 9.6 \times 10^{-4}$) M into a handmade injection valve loaded in a loop (L3). The concentration 4.8×10^{-4} M produced the greatest value of absorbance expressed as peak height in mV (n=3) with high repeatability, as shown in Figure 7-B. The results in Figure 7-C indicated that the best sequence is (D in L1 + O in L2 + R in L3) where D is TIO, R is BPY and O is $FeCl_3$.

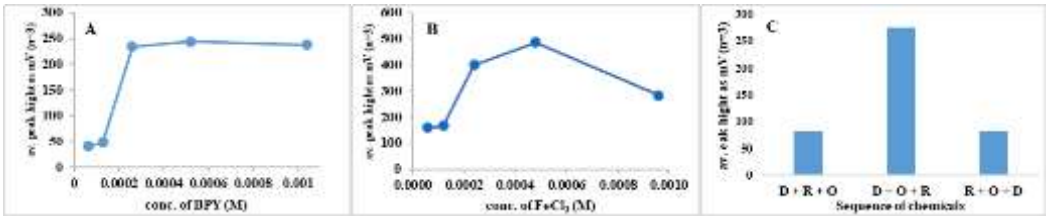


Figure 7: Effect of A\ BPY concentration, B\ $FeCl_3$ concentration, C\ sequence of chemicals. Physical variables

For TIO–BPY- $FeCl_3$ reaction, the best loop volume for drug, oxidized agent and reagent were (117.75-78.50-117.75) µL as shown in Figure 8-A and the best reaction coil length was 50 cm as shown in Figure 8-B. All available flow rates were studied for the system and that shows the best flow rate was 8.1 $mL.min^{-1}$ with sample through-put about 63 samples sec. $hour^{-1}$ as shown in Figure 8-C. The sampling rate was calculated based on the time it took to put the solutions into the seven three-way valve loops (15 sec.) plus the time required to maximum peak height appear (42 sec) so the sampling rate was 63 samples. $hour^{-1}$.

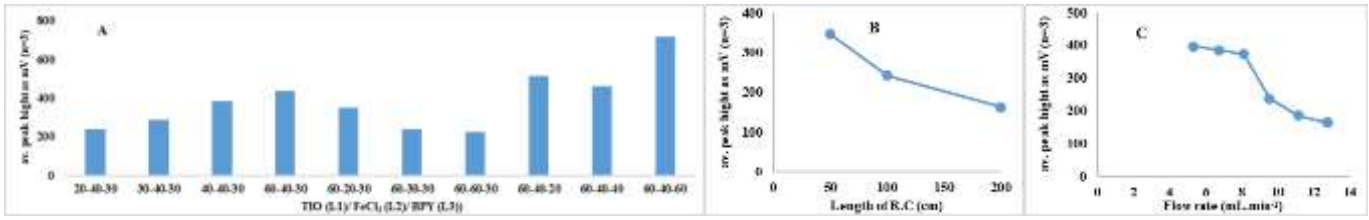


Figure 8: Effect of: A\ Injected volume, B\ Reaction coil, C \ Total flow rate.

Purge time

The purge time for the sample segment that will be transferred into the carrier stream (distilled water) was investigated, using the ideal chemical and physical parameters that were studied previously [28]. For TIO–BPY- $FeCl_3$ time like 5, 10, 15 and 20 sec. and open valve were used, and showed that the purge time was more than 20 sec. giving a highest response intensity. As a reason, the open valve was chosen as the best purge time for completing sample transportation from the sample loop to the flow cell, as shown in Figure 9.

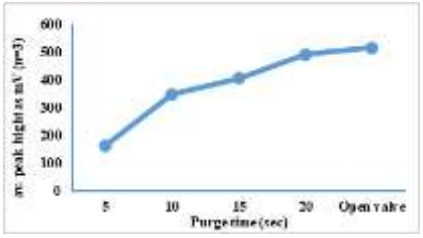


Figure 9: Effect of purge time on peak height in mV for [TIO–BPY- $FeCl_3$] FIA.

Dispersion of zone

Dispersion is a physical phenomenon that happens in the FIA technique as a result of the interaction of different solutions with the sample, which is subsequently dispersed throughout the solution. FIA analytical technique success is based on three concepts [29]. (Reproducible injection time, Reproducible sample injection volume, Control on the dispersion of sample zone). The dispersion of the reaction was 1.32 as shown in Figure 10 and Table 3 The dispersion was calculated according to the law:

D = Co/C. The peak height without dilution (conducting interaction outside the flow injection system) is C_o, while the peak height with dilution is C (conducting interaction inside the flow injection system). In the first experiment, all of the components were combined in an appropriate beaker, and the solution was then sent through the flow injection system (as carrier stream) to get a fixed response expressed (C_o). In the second experiment, TIO into L1, $FeCl_3$ in L2 and BPY in L3. Distilled water is used as a carrier ($mL.min^{-1}$) in the system, and the component injected pushes the components to the reaction coil and subsequently to the detector, resulting in a response represented by (C).

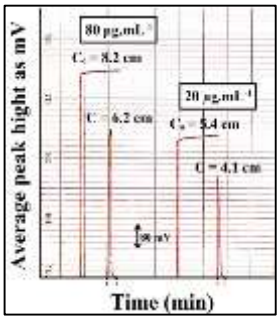


Figure 10: Dispersion of TIO in CFIA system.

Table 3: Dispersion value of TIO.

TIO Conc. µg.mL^{-1}	C_o (cm)	C (cm)	D
20	5.4	4.1	1.32
80	8.2	6.2	1.32

Calibration curve

All ideal conditions after verbal and verified, a series of TIO concentration (from 1 µg.mL^{-1} to 500 µg.mL^{-1}) were prepared and inject to FIA system with $FeCl_3$ and BPY in order to know the optimum range of TIO concentration which can be applicable for this method and it shows that the best concentration range extend (5-200) µg.mL^{-1} as shown in Figure 11 and Table 4.

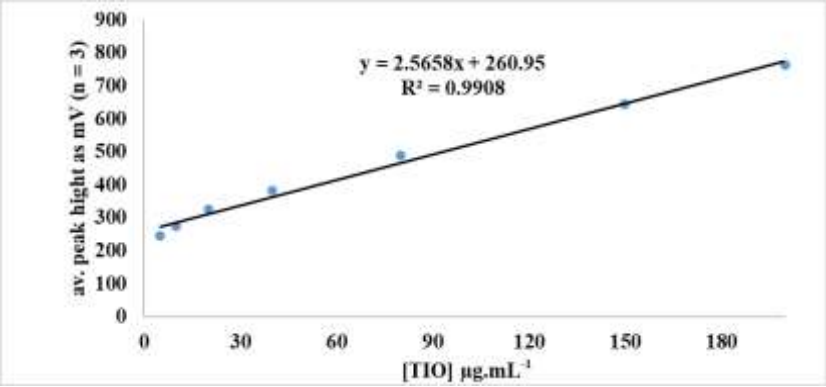


Figure 11: Linear dynamic range for determination of TIO using the developed CFIA system.

Table 4: Calibration table as S.E.M for TIO–BPY- $FeCl_3$.

conc. of TIO (µg.mL^{-1})	peak height (mV)			Average response ($\bar{y}$) (mV)	RSD %	S.E.M	*E/y %
5	240	248	248.8	246	1.98	243±12.08	4.92
10	272	272	276	273	0.84	323±5.73	2.10
20	320	328	328.8	326	1.49	517±12.08	3.71
40	384	384.8	380	383	0.67	683±6.38	1.67
80	480	488	496	488	1.64	795±19.86	4.07
150	640	648	644	644	0.62	947±9.93	1.54
200	760	760	768.8	763	0.67	1123±12.61	1.65

$* \frac{E}{y}\% = t_{tab} \frac{SD}{\sqrt{n}} \times \frac{100\%}{\bar{y}}$, S. E. M $= \bar{x} \mp t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$

Analysis of variation (ANOVA) and Repeatability

Calculate the sum of squares of the difference between the values y_i (response) and $\hat{y}_i$ (appraiser response), (imply error), and call (about regression) for (n_2) freedom degrees [30,31]. Calculate the sum of squares of the variance of values yi from the average value (due to regression) and for one degrees of freedom to get sum of squares $(S_1)^2$, then divide the $(S_1)^2$ on $(S_o)^2$ to get (F), as shown in the Table 5. F_{crit}. (4.7472) << F (28.9728) As a result, it's possible to conclude that there's a direct relationship between TIO concentrations and signal received.

Table 5: ANOVA for the developed FIA technique.

Source of Variation	Sum. of Squares (SS)	df	Mean of Squares (MS)	F $(\frac{S_1^2}{S_2^2})$	F crit
Between Groups (Error) $\sum n_i(\bar{y}_i - \bar{y}_{GM})^2$	1527227.27	1	1527227.27= S^2_2	28.9728	4.7472
Within Groups (Regression) $\sum(n_i - 1)\, S_i^2$	632549.78	12	52712.48= S^2_1		
Total	2159777.05	13			

The repeatability of the suggested FIA method was good as showed in Table 6.

Table 6: Repeatability of consecutive measurement of TIO (n=8).

conc. of TIO (µg.mL^{-1})	Found	Error	Rec%	Erel %	RSD %
20	20.676	0.676	103.379	3.379	1.028
80	80.384	0.384	100.480	0.480	0.557

Methods validation

At the optimized condition, the analytical characteristics of each technique, such as the detection limit, correlation coefficient (r), relative standard deviation, and linear range, were calculated [32,33] as shown in the Table 7. For a set of TIO standard solutions and the suggested method's basic analytical figure of deserts, a calibration curve was constructed (Figure 11). Statistical assessment of regression line presented result of standard deviation for residuals ($S_{y/x}$); slope (S_b) and intercept (S_a) under 95% confidence limits for (n-2) freedom degrees. The small subjects were shown the high repeatability of the results obtained with high reproducibility of the proposed CFIA technique compared with the batch method. Flow injection analysis/merging zones were easier and simpler because that was rapid in analysis (sample throughput of 63 samples. hour^{-1}); large linear scale of calibration curves was obtained.

Table 7: Analytical characteristic of calibration carve for [TIO–BPY- $FeCl_3$] CFIA system.

Parameters	FIA method	Batch method
λ_{max} (nm)	522	522
Regression equation; y = bx+ a; y = absorbance; x = concentration (µg. mL^{-1})	y = 2.5658x + 260.9500	y = 0.0200x + 0.1509
Linear range (µg. mL^{-1})	5 - 200	5 - 40
Average of recovery (Rec%)	101.93	102.91
Average of Relative Error % (Erel%)	1.9297	2.9125
Average of Relative standard deviation (RSD%)	0.7925	0.3620
Slope (b); (mL. µg^{-1}) $b = \frac{\sum_i[(xi-\bar{x})(yi-\bar{y})}{\sum_i(xi-\bar{x})^2}$	2.5658	0.0200
Intercept (a); $a = y - bx$	260.9500	0.1509
Linearity R^2%	99.08	99.73
Correlation coefficient (r): $r = \sum_i[(xi - \bar{x})(yi - \bar{y})]\sqrt{(\sum_i(xi - \bar{x})^2)\,(\sum_i(yi - \bar{y})^2}$	0.9954	0.9986

Standard deviation of slope (Sb) $Sb = \frac{\frac{Sy}{x}}{\sqrt{\sum_i (xi-\overline{x})^2}}$	0.1109	0.0005
Standard deviation of intercept (Sa) $Sa = S_{\frac{y}{x}} \sqrt{n \sum_i (xi - \overline{x})^2}$	11.1660	0.0101
Limit of detection (LOD) : $LOD = \frac{3.3\ SD}{b}$ **and**	4.0670	0.6530
Limit of quantification (LOQ) $LOQ = \frac{10\ SD}{b}$	13.5566	2.1767
Molar absorptivity (Ɛ) (L/mol.cm) $\varepsilon = b \times M.Wt \times 1000$	---------	3259.71
Sandell s sensitivity (S) (µg.cm^{-2}) $S = \frac{M.Wt}{\varepsilon}$	---------	0.0501
Sample through put (h^{-1})	63	5
Standard deviation of the residuals; $S_{\frac{y}{x}} = \sqrt{\frac{\sum_i (yi-\hat{y}_i)^2}{n-2}}$ $\hat{y}_i = bxi + a$	20.6175	0.0146
Confidence limit of slope (b) $CL_b = b \pm t \times Sb$	2.56 ± 0.2716	---------
Confidence limit of intercept (a) $CL_a = a \pm t \times Sa$	260.95 ± 27.3568	---------

Effect of interferences

The interference likely to be introduced by excipients (such as sucrose, cellulose, lactose, glucose, and sodium citrate) was studied in order to check the accuracy of the suggested technique. A sample of pure 100 µg.mL^{-1} TIO spiked with half, equal and double fold excess concentration of selected interferences. The acceptable recovery values demonstrated that there were no interferences during the determination of TIO using new CFIA system, as shown in the Table (8).

Table (8): Interferences effect on [TIO–BPY- $FeCl_3$] FIA system.

Type of Interference	conc. of Interferences (µg.mL^{-1})	Average response ($\overline{y}$) (mV)	Erel%	Rec%
Standard	-----	518	0.0273	100.03
Sucrose	50	525	3.0413	103.04
	100	515	-1.1160	98.88
	200	517	-0.0766	99.92
Cellulose	50	524	2.4177	102.42
	100	512	-2.1553	97.84
	200	520	0.9627	100.96
Lactose	50	520	0.8587	100.86
	100	526	3.1452	103.15
	200	515	-0.8042	99.20
Glucose	50	518	0.1312	100.13
	100	523	2.0020	102.00
	200	513	-1.6356	98.36
Sodium citrate	50	520	1.0666	101.07
	100	518	0.0273	100.03
	200	518	0.3391	100.34

Applications and assessment of suggested method

fore varieties of pharmaceuticals containing TIO have been examined under the suggested approach, which are equipped with distinct sources, according to the conventional addition process. The statistical comparison [25,34] between the proposed method with official Chinese Pharmacopoeia titration method [1] using the student F-test and t-test showed that the calculated F-test values were 1.5889 and 5.5730, t-test values were 0.3104 and 0.4283 less than the theoretical F-test (9.28) and t-test (2.45) via CFIA/MZ. The FIA technique is also successfully used to estimate TIO in a spiked human serum and urine samples. The accuracy

and precision of 100 g.mL^{-1} of TIO were tested. Three times each concentration was examined. Table 10 shows that the serum and urine samples have acceptable reproducibility.

Table (9): Applications of the proposed methods compared with official method for estimation of TIO in medicinal formulations.

Dosage form	Proposed FIA method					Official method (theoretical)				
	conc. of TIO (µg.mL^{-1})		Erel %	Rec %	RSD %	conc. of TIO (µg.mL^{-1})		Erel %	Rec %	RSD %
	Present	Found				Present	Found			
Thiola tablet 100mg, USA	20	20.36	1.80	101.80	1.74	20	20.15	0.75	100.75	1.391
	80	81.02	1.28	101.28	1.19	80	80.12	0.15	100.15	0.512
Tiopronin teva tablet 100 mg, USA	20	19.61	-1.95	98.05	1.80	20	19.72	-1.40	98.60	1.421
	80	79.13	-1.09	98.91	1.22	80	79.56	-0.55	99.45	0.515
Tiopronin Care tablet 100 mg, India	20	19.67	-1.65	98.35	1.80	20	19.70	-1.50	98.5	1.423
	80	80.98	1.22	101.22	1.19	80	80.45	0.56	100.56	0.509
Deutsch-Tiopronin 100 mg, China	20	19.69	-1.55	98.45	1.79	20	19.48	-2.60	97.40	1.439
	80	79.58	-0.52	99.48	1.22	80	79.68	-0.40	99.60	0.514

$t_{tab} = 2.45\ for\ n_1 = n_2 = 4, n_1 + n_2 - 2 = 6, at\ 95\%\ confidence\ level$

$F_{tab} = 9.28\ for\ n_1 - 1 = n_2 - 1 = 3, at\ 95\%\ confidence\ level$

Table 10: Determination of TIO in serum and urine samples using suggest CFIA system.

Serum Samples						Urine Samples					
Sample	**Added Conc. (µ) µg.mL^{-1}**	**Found Conc. ($\bar{x}$) µg.mL^{-1}**	**Erel %**	**Rec. (%)**	**RSD (%)**	**Sample**	**Added Conc. (µ) µg.mL^{-1}**	**Found Conc. ($\bar{x}$) µg.mL^{-1}**	**Erel %**	**Rec. (%)**	**RSD (%)**
1	100	100.86	0.8587	100.86	0.1821	**1**	100	103.04	3.0413	103.04	3.70328
2	100	103.15	3.1452	103.15	0.7877	**2**	100	98.88	-1.1160	98.88	4.70328
3	100	99.20	-0.8042	99.20	0.2688	**3**	100	99.92	-0.0766	99.92	0.46188
4	100	100.13	0.1312	100.13	0.0892	**4**	100	102.42	2.4177	102.42	5.14328
5	100	102.00	2.0020	102.00	0.8837	**5**	100	97.84	-2.1553	97.84	0.92376
6	100	98.36	-1.6356	98.36	1.3496	**6**	100	100.96	0.9627	100.96	5.768882
7	100	101.07	1.0666	101.07	0.2486	**7**	100	100.86	0.8587	100.86	6.466323

Conclusions

After reviewing the literature in the field of injection analysis, it was observed that only some researchers were used this region-based chemical incorporation technique for thiol TIO determination. So, a research plan for our work is suggested a spectrophotometric determination of thiol-sensitive in pure, sampled form doses, urine and serum using a new CFIA design. It has a larger calibration range and a higher sample rate. These procedures can be used to determine the quantity of TIO in $g.mL^{-1}$ without needing to a preceding divorce action, heating or preparation of the specimen, or solid phase extraction. The capital benefit of the methods is a huge workings range; suitable sensitivity and proper for appropriate in routine examination in pharmaceutics specify control laboratories. This is due to their expertness and their result in decrease reagents waste and toxicity of organic reagents when comparison with batch methods and official Chinese Pharmacopoeia titration method.

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