Flow Injection Analysis with Chemiluminescence detection for Determination of Two Phenothiazines

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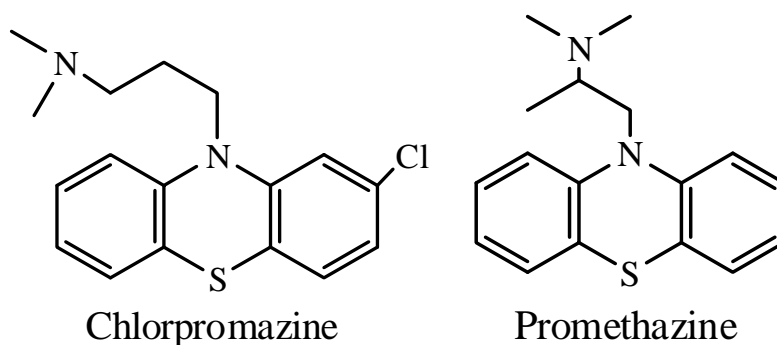* E-mail: Hijran_s77@yahoo.com
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A new flow injection analysis-chemiluminescence (FIA-CL) method for determination of two phenothiazine derivatives "chlorpromazine hydrochloride (CPZ) and promethazine hydrochloride (PTZ)" described. The redox reaction between Fe(III) and phenothiazines was taken as a base of this new method. A well-defined volume of CPZ or PTZ and luminol solution is injected simultaneously by means of two injection valves in accordance with the merging zone principle. Calibration graphs were constructed in the range of (0.05 – 1.4μg/ml) for CPZ with correlation coefficient of (0.9972) and in the range of (0.5 – 40μg/ml) for PTZ with correlation coefficient of (0.9978). The precision and accuracy of the method were checked by calculation of relative standard deviation (RSD) and relative error percentage (%E) for two different levels of concentrations. The method was relatively free from common excipients and it is applied successfully for the determination of CPZ and PTZ in pharmaceutical formulations. The results obtained were in good agreement with those obtained by a reference method in British Pharmacopoeia.

Key Words: Flow injection, Chemiluminescence, Phenothiazines, Pharmaceutical analysis

1. Introduction

Phenothiazines are an important group of neuroleptics used in the treatment of moderate and severe mental and emotional conditions. They are also known for their antiemetic effects, the potency of the effects of anaesthetics, analgesics and sedatives and also as antihistamines. More than 100 compounds derived from the fundamental phenothiazine skeleton have been synthesized and pharmacologically tested in the past four decades[1]. Two of phenothiazine derivatives have been studied here: chlorpromazine (CPZ) "[3-(2-chlorophenothiazine-10-yl)propyl]dimethyl amine" and promethazine (PTZ) or [N,N-α-trimethyl-10H-phenothiazine-10-ethanamine][2], their chemical structures are shown in Fig. (1).

Chlorpromazine Promethazine

The oxidation of 2,10-disubstituted phenothiazines involves a series of one-electron steps providing free radicals and cations[3]. Many methods have been reported for the individual determination of these two compounds, including those involving spectrophotometry[4-15], colorimetry[16], HPLC[17-19], titrimetry[20-22], and spectrofluorimetry[23, 24]. Electrochemical methods have been recently applied for the analysis of these drugs[25-27].

Chemiluminescence (CL) method used for the determination of CPZ[28 – 33] and PTZ[34, 35]. In 1994, Bendito's group[29] described a CL method for chlorpromazine hydrochloride based on the hydrogen peroxide – bis(2,4,6-trichlorophenyl)oxalate system. In order to alleviate the high background emission in the peroxyoxlate chemiluminescence reaction, they used the continuous addition of reagent technique, for which the limit of detection for chlorpromazine hydrochloride was 0.0665μg/ml.

A CL flow system is described for the determination of promethazine hydrochloride based on its direct oxidation with cerium(IV) in acidic media accompanied with CL. The system responds linearly to promethazine

hydrochloride concentration in the range 0.15 – 10 µg/ml [34]. Salah M. Sultan et al. [35] described a specific and highly sensitive flow injection method for the determination of promethazine hydrochloride. The method was based on the CL emission intensity produced as a result of its oxidation reaction with permanganate in sulfuric acid medium. The method was found to be applicable in the concentration range of promethazine hydrochloride 5.0 – 600 µg/ml. The hemiluminescence from the oxidation of luminol by hydrogen peroxide in alkaline medium has, so far, been the most attractive system for determining many inorganic and organic species [36].

In the present work two phenothiazines (chlorpromazine and promethazine) were determined by FIA-CL method depending on merging zone principle using Luminol-H_2O_2-Fe(III) system, while the CL intensity increased by increasing the phenothiazines concentrations during oxidation of phenothiazines.

2. Experimental

2.1. Apparatus:

The schematic diagram of the FI-CL system used in this work is shown in Fig. (1). It consists of a peristaltic pump (DESAGA Heidelberg, with 6 channels and variable speed up to 10 ml/min) to deliver flow streams. Two rotary valves (Rheodyne U.S.A.) with variable sample volumes were used to inject the sample and reagent into flowing carrier streams. A Y-shaped perspex piece was used to mix two streams of reagents.

The flow cell that used for the present work was made by winding the length of glass tubing (0.8 mm i.d) to form coil of 100 µL volume. At the entrance of the cell the reagent and luminol are mixed to produce CL. The mixing position of the flow cell was considered on the detector inside the spectrophotometer (Type CECIL CE303) the light source of which was blocked. D.C-microvoltmeter type (PHILIPS PM 2434) was used as associated electronics. The chemiluminescent out-put was recorded by mean of x-t recorder (Type PM 825A PHILIPS – one line recorder) with various amplication factors and different chart speeds.
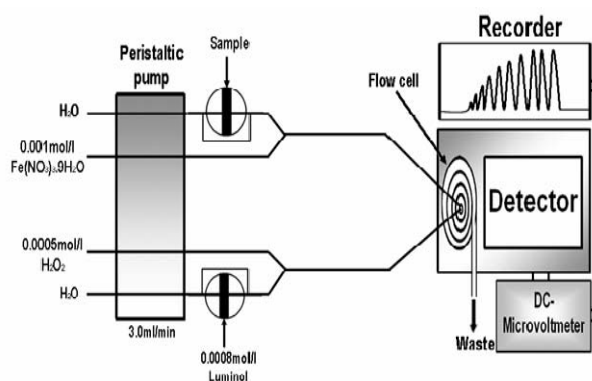

Fig. (1): Schematic diagram of the FIA-CL manifold used for the determination of CPZ and PTZ.

2.2. Reagents:

All chemicals and reagents used were of analytical or pharmaceutical grade. Distilled water was used for the preparation of all solutions. Sodium carbonate (Fluka), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Surechem - LTD), ferric nitrate (Fluka), hydrogen peroxide (Gainland Chemical Company U. K. - GCC) and all interfering species were obtained from local sources.

Stock solutions of CPZ and PTZ

A stock solution (100µg/ml) of each CPZ-HCl (Summara drug industry – S. D. I.) and PTZ-HCl (S.D.I.) was prepared daily by dissolving 0.1 g of a pure compound in distilled water and diluting to 1.0L in amber-colored volumetric flask and stored in the dark. A series of standards were prepared by diluting appropriate amounts of each stock solution with distilled water.

Sample preparation

Tablets: Twenty tablets of each commercial sample were weighed then ground to a fine powder and mixed. A sample equivalent to approximately 100 mg preparations was weighed accurately and dissolved in a beaker, the solution transferred quantitatively to a 100 ml volumetric flask and diluted with distilled water to the 100 ml mark. The mixture was shaken mechanically (using Lab. Companion Shaking water bath BS-06) for 15 minutes and then filtered. A 10 ml portion of the filterate was transferred into 100 ml volumetric flask and diluted to volume with distilled water. As the proposed method, the sample was then diluted to the concentration with the calibration range.

Syrup: 10 ml of syrup was diluted to 100 ml with distilled water in a volumetric flask. A 25 ml volume of the solution was transferred into 100 ml calibrated flask and diluted to the mark with distilled water.

2.3. General procedure:

The FI-CL system in Fig. (1) was operated. The procedure includes mixing of two main streams which merges in a controlled manner. In the first stream a 90 μL of CPZ-HCL or PTZ-HCl is injected into the carrier stream (H_2O) which then mixed with Fe(III) stream, while in the second stream a 90 μL portion of luminol solution was injected to the carrier H_2O stream that merged with hydrogen peroxide stream before reaching the flow cell. Both luminol and the drug were injected simultaneously according to merging zone principle so that the center of the both sample and reagent slugs match each other at the confluence point inside the flow coil which give rise to CL emission signal. The CL signal produced in the flow cell was detected and recorded. The concentration of CPZ and PTZ was quantified by CL emission intensity. A calibration graph of CL intensity (mV) versus concentration constructed.

3. Results and discussion

3.1. Optimization of experimental parameters

All chemicals and physical conditions that participate in the reaction have been studied to obtain maximum sensitivity of the CL intensity. These studies started using the following experimental chemical and physical variables, 1.0×10^{-3} mol/l Fe(III), 5.0×10^{-4} mol/l H_2O_2, 8.0×10^{-4} mol/l luminol, 3.0 ml/min flow rate and 90 μL sample volume. It was necessary to perform optimizations in the presence of the phenothiazines because of using two injection valves according to merging zone principle which results in different experimental conditions.

3.1.1. Chemical Optimizations

Effect of Fe(III) concentration

In order to ascertain the optimum CL conditions, several Fe(III) compounds were tested to investigate the most useful compound for the determination of CPZ and PTZ. These compounds include $FeCl_3$, $Fe_2(SO_4)_3$ and $Fe(NO_3)_3.9H_2O$. The latter gave the most satisfactory results, and hence it was chosen for further work.

The effect of Fe(III) concentration on the CL intensity was carried out using concentration ranges of Fe(III) $5.0 \times 10^{-4} - 1.0 \times 10^{-2}$ mol/l using preliminary conditions mentioned in section (2.4.1), 0.8 μg/ml CPZ and 16.0 μg/ml PTZ; the results are given in Fig. (2). The maximum CL intensity was obtained at a Fe(III) concentration of 5.0×10^{-3} mol/l. When the Fe(III) concentration was higher than this level, the CL intensity decreased, mainly due to the appearance of intense colored product between Fe(III) and CPZ or PTZ. Thus, 5.0×10^{-3} mol/l Fe(III) was chosen as being the most suitable for further studies.
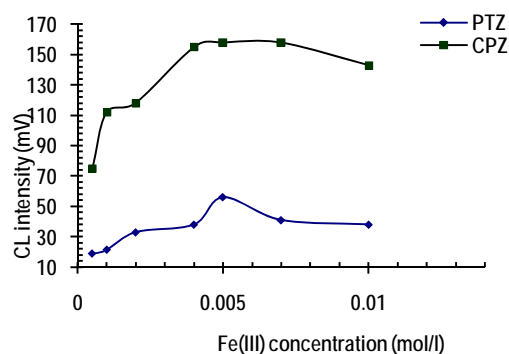

Fig. (2): Effect of Fe(III) concentration on the CL intensity of 0.8μg/ml CPZ and 16.0 μg/ml of PTZ.

Effect of H_2O_2 concentration

Keeping Fe(III) concentration at the optimum value, the influence of the hydrogen peroxide concentration on the CL intensity was investigated in the range of $3.0 \times 10^{-4} - 1.0 \times 10^{-2}$ mol/l. Fig. (3) shows that the maximum increase in the CL intensity was obtained as the concentration of H_2O_2 increased up to 5.0×10^{-3} mol/l. Above 5.0×10^{-3} mol/l H_2O_2, the CL intensity decreased. Therefore, 5.0×10^{-3} mol/l of the H_2O_2 concentration was selected to be the optimum and used for subsequent experiments.
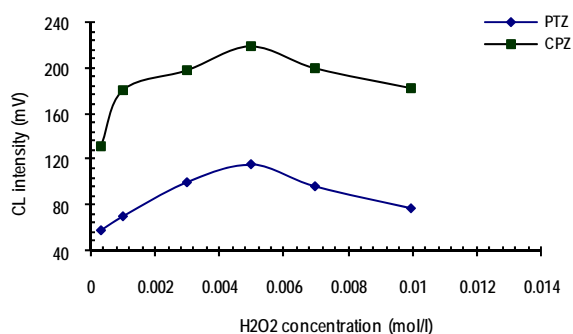
Fig. (3): Effect of H_2O_2 concentration on the CL intensity of 0.8μg/ml CPZ and 16.0 μg/ml of PTZ.

Effect of Luminol concentration

The influence of the luminol concentration on the CL intensity was investigated in the range of 1.0×10^{-4} - 2.0×10^{-3} mol/L. Fig. (4) illustrates that the maximum increase in the CL intensity was obtained at 1.0×10^{-3} mol/L. Thus, 1.0×10^{-3} mol/L luminol was used for further work.
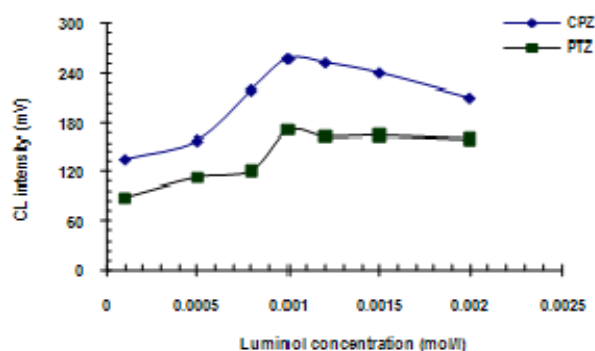

Fig. (4): Effect of luminol concentration on the CL intensity of 0.8μg/ml CPZ and 16.0 μg/ml of PTZ.

3.1.2. Physical optimizations

Effect of using mixing coil

The effect of mixing coil length in the range of (0 – 50 cm) for mixing of samples with Fe(III) on the CL intensity was studied with constant internal diameter of 0.8mm. At the same time a coil with the same length put into after position of mixing of luminol with H_2O_2 to provide a suitable length for the merging zone mode. Fig. (5) shows a continuous decrease in CL intensity as length of the mixing coil increase, this indicate that the reaction is fast and does not require more time.
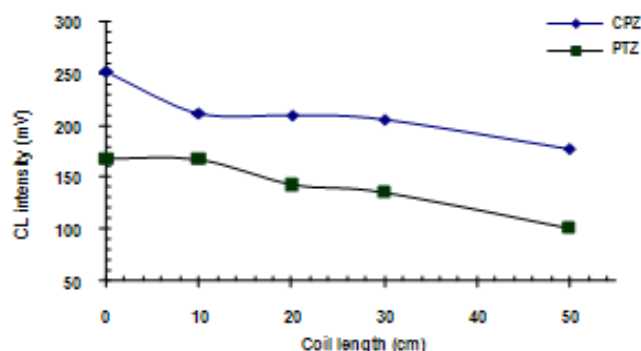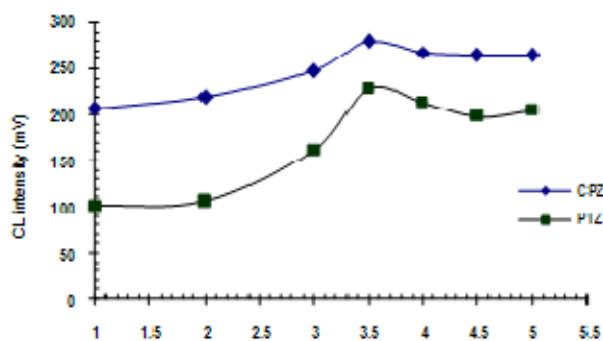

Fig. (5): Effect of using mixing coil on the CL intensity.

Effect of flow rate

The effect of the flow rate on the intensity of the CL system was studied in the range 1.0 – 5.0 ml/min. As shown in Fig. (6) a flow rate of 3.5 ml/min give higher CL intensity.

Fig. (6): Effect of flow rate on the CL intensity of 0.8μg/ml CPZ and 16.0μg/ml of PTZ.

Effect of sample volume

Using all experimental chemical and physical parameters at their optimized values, the variation in the CL intensity with the injected sample volume (loop) in the range 50-150μL were studied.

The results in Fig. (7) shows that there is an increase in the CL intensity up to 100μL, above that the CL intensity decreases due to formation of intense color that obscure the CL light intensity. Therefore, sample volume of 100μL was selected as optimum for the present method.
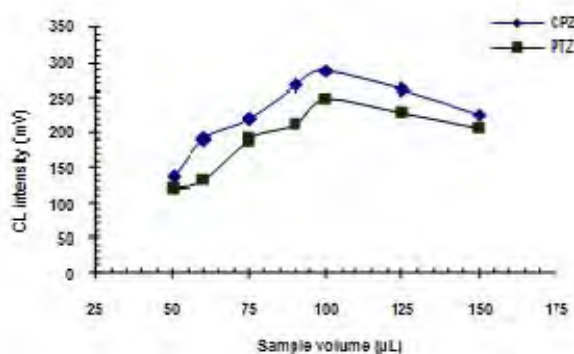

Fig. (7): Effect of sample volume on the CL intensity.

Table (1) illustrates summary of optimum chemical and physical conditions for the determination of chlorpromazine hydrochloride and promethazine hydrochloride using FIA-CL system.

Table (1): Summary of optimum chemical and physical conditions for the determination of CPZ and PTZ.

Parameters	Optimum value
Ferric nitrate	5.0×10^{-3} mol/l
Hydrogen peroxide	5.0×10^{-3} mol/l
Luminol	1.0×10^{-3} mol/l
Flow rate	3.5 ml/min
Sample volume	100 μL

4. Calibration graph

The FIA-CL system shown in Fig. (1) was operated using the optimum experimental conditions in Table (1). Calibration graphs in the range of 0.05 – 1.4 μg/ml CPZ.HCl and 0.5 – 40μg/ml PTZ.HCl were obtained as shown in Fig. (8 and 9) respectively. Statistical treatments of the calibration results are shown in Table (2). To determine the accuracy and precision of the proposed method, ten replicate determinations were made on the two different concentrations of standard CPZ and PTZ solutions. The accuracy was checked with a relative error (%E), while the precision of the method is checked with a relative standard deviation (RSD) of the same solutions. The results are shown in Table (3) which indicates good accuracy and precision.
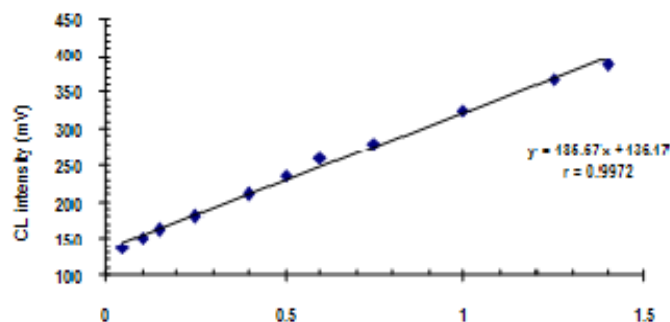
Fig. (8): Calibration graph for the determination of Chlorpromazine.HCl using FIA-CL system.
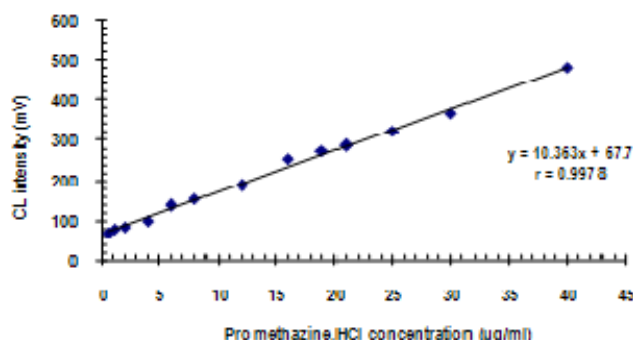

Fig. (9): Calibration graph for the determination of Promethazine.HCl using FIA-CL system.

Table (2): Analytical characteristics for the determination of CPZ and PTZ.

Drug	Linear range (µg/ml)	D.L.* (µg/ml)	r**	n***
CPZ-HCl	0.05 – 1.4	0.01	0.9972	11
PTZ-HCl	0.5 – 40	0.2	0.9978	13

* Detection limit, ** Correlation coefficient, and *** Number of measurements.

Table (3): Accuracy and precision of the method.

Drug	Conc. of drug (µg/ml)	Mean*	%E	SD	RSD%
CPZ	0.6	245	-2.30	2.45	0.99
	1.0	320.5	-0.72	1.35	0.42
PTZ	7.0	137.3	-4.05	2.71	1.97
	22	291.5	-1.83	3.10	1.06

* Mean of 10 injections, (%E) is relative error, (S.D.) is standard deviation and (R.S.D.) is relative standard deviation.

5. Interferences

Effects of some common foreign species which can be found in typical pharmaceutical preparations were examined for the determination of CPZ-HCl and PTZ-HCl. The effects were calculated by comparing the CL intensity (mV) obtained with solutions containing 0.25µg/ml CPZ or 7.0µg/ml PTZ and different concentrations of the other compounds (interferences) with that obtained by injecting aqueous solutions of CPZ (0.25µg/ml) and PTZ (7.0µg/ml). Table (4) shows maximum tolerable concentrations of the various compounds. Therefore, the method can be applied selectively for determination of CPZ and PTZ in their pharmaceutical dosage forms.

Table (4): Effect of interference on the CL intensity of 0.25μg/ml CPZ and 7.0μg/ml PTZ.

Interference	Chlorpromazine.HCl			Promethazine.HCl		
	Conc. of interference (μg/ml)	CL intensity (mV)	%E	Conc. of interference (μg/ml)	CL intensity (mV)	%E
Glucose	5.0	182.5	-0.18	140	140.5	0.35
Sucrose	5.0	181.5	-2.34	140	139.66	-0.79
Galactose	5.0	182.5	-0.18	140	140.5	0.35
Lactose	5.0	182	-1.26	140	141	1.04
Fructose	5.0	180.5	-4.49	140	138.5	-2.4
Starch	5.0	182	-1.26	140	139.75	-0.67
Magnesium streate	1.25	183.5	1.96	35	142.5	3.11
Mixture of interferences	5.0	183	0.88	140	141	1.04

6. Applications

The new method is applied for the determination of CPZ and PTZ in several commercial pharmaceutical preparations. In every case, the official method of British pharmacopoeia [2] was used as a reference method including UV-spectrophotometric method. The results obtained are included in Table (5). The results of two methods are compared using the F-test and t-test. From the values of F-calculated (4.86) of the experiment and F-value from the table (6.39) [37] with a confidence limit of 95%, and the value of t_{exp}= 0.3469 and t_{table} = 2.78 with a confidence limit of 95% the results indicated that there is no significant difference between the precision and accuracy of the two methods.

Table (5): Determination of CPZ and PTZ in commercial drug formulations with the proposed and the standard methods.

Formulation	Company	Composition	Amount nominal (mg per tablet or ml)	Drugs found (mg) per tablet or ml		%E
				Proposed method [*]	Standard method	
Chlorpromazine (tablet)	MEDICO REMEDIES PVT. LTD – Palghar, marketed by LGT (U. K.) LTD.	Chlorpromazine hydrochloride	100	100.66	100.30	0.3614
Chlorpromazine (tablet)	Tehran Chemie pharmaceutical Co. - Tehran-Iran	Chlorpromazine hydrochloride	100	97.32	99.13	-1.817
Largactil (tablet)	OUBARI PHARMA – Aleppo – Syrie under license from RHONE – POULENC RORER - France	Chlorpromazine hydrochloride	100	102.71	101.47	1.2262
Promethazine (tablet)	Tehran Chemie pharmaceutical Co. - Tehran-Iran	Promethazine hydrochloride	25	25.48	25.29	1.2571
Promethazine (syrup)	Sammara drug industry (S.D.I.) - Iraq	Promethazine hydrochloride	5	5.0573	5.01	0.8828

[*] Average of three measurements (n=3)

7. Conclusions

A flow injection chemiluminescence method was proposed for determination of CPZ.HCl and PTZ.HCl based on enhancement of Luminol-Hydrogen peroxide catalyzed Fe(III) system by CPZ or PTZ. The proposed method is simple, precise, accurate, sensitive enough to permit the determination of CPZ and PTZ in pharmaceutical formulations and free from many disadvantages that are common in spectrophotometric methods: complex sample treatment, critical working conditions, heating of the reaction mixture, expensive chemicals and instrumentation and high time consuming, etc.

The sensitivity of this method is not less than that obtained for determination CPZ using spectrophotometric [12], FIA-CL [33] or FIA-potentiometric [38] methods that gives linear ranges between 1.0–15, 0.05–10 and 3.55–

3553µg/ml respectively. The proposed method is less time consuming (about 35 sec/sample) comparing with electrochemiluminescent method [31] that give linear range between 0.003–1.066µg/ml and need about 10 minutes for total analysis, while spectrophotometric determination of PTZ give linear range (4 – 28µg/ml) and the color reached its maximum intensity after five minutes [39]. Therefore it is useful for routine analysis of the CPZ and PTZ in pharmaceutical formulations. In addition, it decreases the possibility of interference caused by common foreign species.

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