

## Flow-Injection Chemiluminometric Determination of Pioglitazone HCl by Its Sensitizing Effect on the Cerium-Sulfite Reaction

Nawal A. AL-ARFAJ, Eman A. AL-ABDULKAREEM, and Fatma A. ALY†

Department of Chemistry, College of Science, Women Student—Medical Studies and Science Sections,  
King Saud University, P. O. Box 22452, Riyadh 11495, Saudi Arabia

A flow injection chemiluminescent (FI-CL) method was developed for the determination of pioglitazone HCl. It is based on the sensitizing effect of the drug on the oxidation reaction of sulfite with cerium(IV). The different experimental parameters affecting the chemiluminescence intensity, such as concentration of reagents and some physical parameters of the manifold, were carefully studied and incorporated into the procedure. The method permits the determination of 0.05 – 3.0  $\mu\text{g ml}^{-1}$  of pioglitazone HCl with correlation coefficient  $r = 0.9999$ . The lower limit of detection (LOD) is 0.01  $\mu\text{g ml}^{-1}$  ( $S/N = 2$ ) and the lower limit of quantitation (LOQ) is 0.05  $\mu\text{g ml}^{-1}$ . The proposed method was compared with other reported methods and was found to be equally accurate and precise. It was successfully applied to the determination of the drug in pharmaceutical preparations and in biological fluids.

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### Introduction

Pioglitazone HCl (( $\pm$ )-5-[4-[2-(5-ethyl-2-pyridinyl)ethoxy]-phenyl]-2,4-thiazolidinedione) hydrochloride (Fig. 1) is an oral hypoglycemic agent that acts primarily by increasing insulin sensitivity in target tissues. It is used both as monotherapy and in combination with sulfonylurea or insulin in the management of type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus).<sup>1–3</sup>

Several analytical methods have been reported for the determination of pioglitazone HCl in bulk form, pharmaceuticals and biological fluids. Most of the reported methods are chromatographic methods and no official methods have been reported for the determination of pioglitazone HCl. The reported methods include: high performance liquid chromatography with ultraviolet detection (HPLC-UV),<sup>4,8</sup> high performance liquid chromatography with mass spectroscopy (HPLC/MS),<sup>9</sup> high performance liquid chromatography with tandem mass spectroscopy (HPLC/MS/MS),<sup>10,11</sup> high performance thin layer chromatography (HPTLC),<sup>12</sup> thin layer chromatography (TLC),<sup>13,14</sup> micellar electrokinetic chromatography (MEKC)<sup>15</sup> and capillary electrophoresis method (CE).<sup>16,17</sup> Other reported methods include a potentiometric method<sup>18</sup> and UV spectrophotometric methods.<sup>19,20</sup>

The chemiluminescence methods are generally highly sensitive, because low light levels are readily monitored in the absence of noise. Furthermore, radiation attenuation by a filter or monochromator is avoided. In fact, detection limits are usually determined not by the detector sensitivity but rather by the reagents' purity. CL methods have many advantages such as low cost instrumentation, since it is homemade; low detection limits; large calibration ranges; and short analysis time. So the

CL methods have allowed many analysts to develop a huge number of CL systems in the last half century.

The oxidation of sulfite by Ce(IV) in sulfuric acid medium is a well-known chemiluminescence reaction.<sup>21–24</sup> The emission has been attributed to the formation of excited sulfur dioxide molecules which radiate during de-excitation.<sup>25</sup>

This paper describes the chemiluminescence properties of the system Ce(IV)-sulfite-pioglitazone HCl. The optimized procedure was applied to the determination of pioglitazone HCl in both pharmaceuticals and spiked biological fluids. Our reviewing the literature revealed that, up to the present time, nothing has been published concerning the chemiluminescence determination of pioglitazone HCl.

### Experimental

#### Apparatus and flow system

The flow system used for the determination of pioglitazone HCl with CL detection is shown schematically in Fig. 2. A 3MP4 peristaltic pump (Gilson Minipuls) with two channels and variable speed was used to drive the carrier and the reagent streams through the flow system. Each stream was pumped at a constant flow rate of 3  $\text{ml min}^{-1}$  using PTFE tubing (0.06 mm i.d.). Pioglitazone HCl solutions were injected through the sample injection valve; this procedure allows mixing of the sample with sulfite solution and then combination with Ce(IV) solution just before the detector. The emitted light was

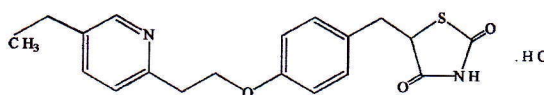


Fig. 1 Structure of pioglitazone HCl.

† To whom correspondence should be addressed.  
E-mail: elzahraa@ksu.edu.sa



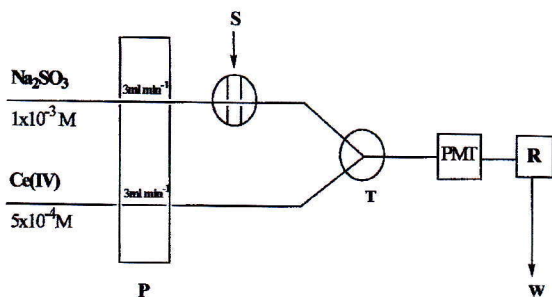


Fig. 2 FI manifold for the chemiluminescence determination of pioglitazone HCl: P, peristaltic pump; S, sample port; T, perspex T-piece; PMT, photomultiplier tube; R, recorder; W, waste.

measured by a photomultiplier tube (Thorn EMI, 9789QB) which was operated at 1100 V. The signal was recorded by a (Chessell Ltd.) recorder. Peak heights were measured.

#### Reagents and materials

A stock standard solution of pioglitazone HCl (Chargen-Zert, Germany)  $1.0 \text{ mg ml}^{-1}$  was prepared in 0.1 M hydrochloric acid (BDH Ltd., UK), which was stable for at least one week in the refrigerator. Working standard solutions of pioglitazone-HCl were prepared by dilution with distilled water immediately before use. Other reagents used were: aqueous sodium sulfite (BDH Ltd., UK),  $1 \times 10^{-3} \text{ M}$ ; cerium ammonium sulfate dihydrate solution (Fluka),  $5 \times 10^{-4} \text{ M}$ ; and sulfuric acid solution (BDH Ltd., UK),  $5 \times 10^{-3} \text{ M}$ .

#### Procedures

**General procedure.** Working solutions of pioglitazone HCl in the range of  $0.05 - 3.0 \text{ } \mu\text{g ml}^{-1}$  (Table 1) were prepared from the stock solution. A  $450 \text{ } \mu\text{l}$  aliquot of pioglitazone HCl was injected into a stream of  $1 \times 10^{-3} \text{ M}$  sodium sulfite solution; the mixture was then combined with a stream of  $5 \times 10^{-4} \text{ M}$  Ce(IV) solution and the resulting peak heights were measured. Calibration graphs were prepared by plotting the peak heights against the drug concentration.

**Procedure for tablets.** An accurately weighed amount of ten powdered tablets, equivalent to  $10.0 \text{ mg}$  of the drug, was transferred into a  $50 \text{ ml}$  volumetric flask. Then  $0.1 \text{ M}$  hydrochloric acid was added to the flask and the volume was completed to the mark. The flask with its contents were sonicated for  $20 \text{ min}$  and then the contents were filtered. Proceed as described in the general procedure. The nominal content was calculated either from the previously plotted calibration graph or using the corresponding regression equation.

**Procedure for spiked biological fluids.** Aliquots of urine or serum ( $1.0 \text{ ml}$ ) in six centrifuge tubes were spiked with aliquots of aqueous solutions of pioglitazone HCl containing  $1, 3, 5, 10, 15$  and  $20 \text{ } \mu\text{g}$  and the tubes were vortexed for  $1 \text{ min}$ . Next,  $0.2 \text{ ml}$  of disodium tetraborate solution was added to each tube, and then each tube was vortexed for  $1 \text{ min}$ . Here,  $5 \text{ ml}$  of dichloromethane was added to each tube and the tubes were shaken for  $10 \text{ min}$ . The tubes were then centrifuged at  $3000 \text{ rpm}$  for  $10 \text{ min}$  at room temperature. The resulting organic layers were collected and the extraction was repeated for each tube two times with  $5 \text{ ml}$  of dichloromethane. The combined extracts were evaporated to dryness at room temperature and the residues were dissolved in  $1 \text{ ml}$  of  $0.1 \text{ M}$  HCl. Each solution was transferred into a  $10 \text{ ml}$  volumetric flask and completed to volume with distilled water, then analyzed according to the

Table 1 Analysis of pioglitazone HCl in its pure and dosage forms by the proposed and the published spectrophotometric<sup>20</sup> methods

Preparation	Taken/ $\mu\text{g ml}^{-1}$	Found, %	
		Proposed method <sup>b</sup>	Published method <sup>20</sup>
Pure form	0.05	99.7	
	0.2	100.0	
	1.0	100.8	
	2.0	100.0	
	2.5	99.6	
	Mean $\pm$ SD	100.0 $\pm$ 0.47	99.4 $\pm$ 0.46 <sup>e</sup>
Actos tablets <sup>a</sup> (15 mg pioglitazone HCl/tablet)	Student's <i>t</i> -value	1.904 (2.44) <sup>c</sup>	
	Variance ratio	1.044 (19.2) <sup>d</sup>	
	0.2	98.9	
	0.5	101.0	
	1.0	100.7	
	1.5	98.3	
Actos tablets <sup>a</sup> (30 mg pioglitazone HCl/tablet)	2.0	98.0	
	Mean $\pm$ SD	99.4 $\pm$ 1.38	100.6 $\pm$ 1.44 <sup>e</sup>
	Student's <i>t</i> -value	1.05 (2.44) <sup>c</sup>	
	Variance ratio	1.09 (6.94) <sup>d</sup>	
	0.2	98.9	
	0.5	99.1	
Actos tablets <sup>a</sup> (15 mg pioglitazone HCl/tablet)	1.0	99.3	
	1.5	101.7	
	2.0	98.7	
	Mean $\pm$ SD	99.6 $\pm$ 1.23	99.7 $\pm$ 1.53 <sup>e</sup>
	Student's <i>t</i> -value	0.133 (2.44) <sup>c</sup>	
	Variance ratio	1.55 (6.94) <sup>d</sup>	

a. Products of Takeda Chemical Industries Ltd., Osaka, Japan. Batch numbers for Actos 15 mg (656185) and for Actos 30 mg (656165).

b. Each result is the average of three separate determinations.

c. Tabulated *t*-value at confidence levels 95%.<sup>30</sup>

d. Tabulated *F*-value at confidence levels 95%.<sup>30</sup>

e. For three different determinations.

general procedure. A blank experiment was carried out adopting the above procedure.

## Results and Discussion

The sensitizing effect of pioglitazone HCl on the chemiluminescent reaction of the oxidation of sodium sulfite was studied using different oxidants. A very weak CL was obtained with potassium permanganate. Other oxidants, such as potassium dichromate and potassium bromate, gave good CL signals, but maximum CL intensity was obtained when Ce(IV) was used as an oxidant in an acidic medium.

A weak CL signal (recorded as a baseline) appeared when solutions of sodium sulfite and Ce(IV) were mixed and flowed in the FIA system. This signal notably increased when pioglitazone HCl was injected, and gave a peak whose height increased proportionally with pioglitazone HCl concentration. Maximum CL signal was obtained when the sample was injected into a stream of  $1 \times 10^{-3} \text{ M}$  sodium sulfite and then mixed with  $5 \times 10^{-4} \text{ M}$  Ce(IV) prior to the detector. Various chemical and instrumental parameters affecting the CL intensity were investigated and optimized for the pioglitazone HCl determination.



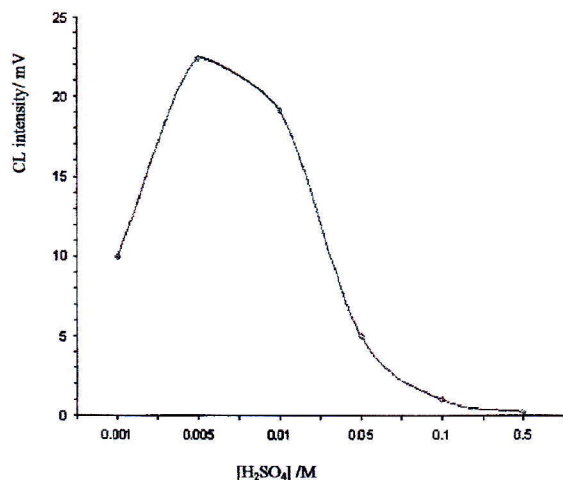


Fig. 3 Effect of  $\text{H}_2\text{SO}_4$  concentration as a solvent for  $\text{Ce(IV)}$  on CL intensity of pioglitazone HCl. Injected drug solution,  $1.0 \mu\text{g ml}^{-1}$ ;  $[\text{Ce(IV)}] = 5 \times 10^{-4} \text{ M}$ ;  $[\text{Na}_2\text{SO}_3] = 1 \times 10^{-3} \text{ M}$ ; loop size,  $450 \mu\text{l}$ .

#### Configuration designs

The flow injection configuration used for the determination of pioglitazone HCl, was designed so as to provide different reaction conditions for magnifying the CL signal generated by the reaction. A CL signal was obtained only when the sample was injected into a stream of  $1 \times 10^{-3} \text{ M}$  sodium sulfite and then mixed with  $5 \times 10^{-4} \text{ M}$   $\text{Ce(IV)}$  prior to the detector.

$\text{Ce(IV)}$  and sodium sulfite solutions are continuously mixed and introduced into the flow cell and the weak CL radiation emitted from this reaction is continuously recorded as the baseline. When a pioglitazone HCl solution is injected into the sodium sulfite stream, the intensity is enhanced in proportion to its concentration. A schematic diagram of the manifold is shown in Fig. 2.

#### Optimization of experimental variables

The effect of experimental conditions on the CL intensity was studied. A series of experiments were conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and some physical variables, including the flow rate, the sample volume and the coil length from T-piece to PMT.

##### Effect of sulfuric acid concentration as a solvent for $\text{Ce(IV)}$

The  $\text{Ce(IV)}$  becomes stable when dissolved in sulfuric acid solution, so the effect of sulfuric acid concentration on the CL emission was studied in the range  $1 \times 10^{-3}$  to  $0.5 \text{ M}$  (Fig. 3). The highest CL emission was obtained at  $5 \times 10^{-3} \text{ M}$  sulfuric acid; thus, this concentration was used in the preparation of  $\text{Ce(IV)}$  solutions.

##### Effect of $\text{Ce(IV)}$ concentration

The CL intensity depends on  $\text{Ce(IV)}$  concentration, so a study was carried out in the range  $1 \times 10^{-5}$  to  $5 \times 10^{-2} \text{ M}$  (Fig. 4). The maximum CL signal was obtained with  $5 \times 10^{-4} \text{ M}$   $\text{Ce(IV)}$ , so this value was used for further investigation.

##### Effect of sodium sulfite concentration

The CL intensity was studied in the range of  $1 \times 10^{-5}$  up to  $1 \times 10^{-2} \text{ M}$ . The optimum sulfite concentration was  $1 \times 10^{-3} \text{ M}$  after which the intensity started to decrease. Figure 4 shows the effect of sodium sulfite concentrations on the CL intensity.

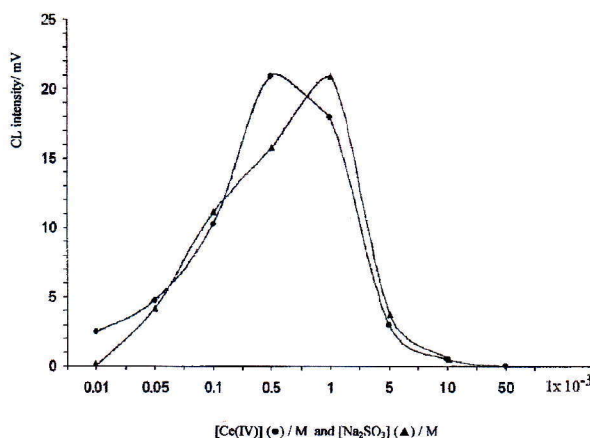


Fig. 4 Effect of  $\text{Ce(IV)}$  concentration (●) and  $\text{Na}_2\text{SO}_3$  concentration (▲) on CL intensity of pioglitazone HCl. Injected drug solution,  $1.0 \mu\text{g ml}^{-1}$ ; loop size,  $450 \mu\text{l}$ .

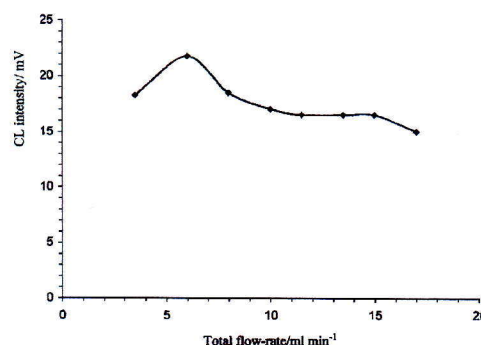


Fig. 5 Effect of total flow-rate on CL intensity of pioglitazone HCl. Injected drug solution,  $1.0 \mu\text{g ml}^{-1}$ ;  $[\text{Ce(IV)}] = 5 \times 10^{-4} \text{ M}$ ;  $[\text{Na}_2\text{SO}_3] = 1 \times 10^{-3} \text{ M}$ ; loop size,  $450 \mu\text{l}$ .

##### Effect of total flow rate

Once the concentrations of reagents were optimized, the effect of the flow rate was studied, the solutions of oxidant and sulfite were introduced into the manifold at equal flow rates. The effect of total flow rate on the CL intensity is shown in Fig. 5. The optimum intensity was obtained on using a total flow rate of  $6 \text{ ml min}^{-1}$ .

##### Effect of sample volume

The injected sample volume was studied by using different loop sizes in the range  $10 - 700 \mu\text{l}$ . The results in Fig. 6 showed an increase in the CL intensity up to  $450 \mu\text{l}$ , above which the intensity was almost constant.

##### Effect of coil length from T to PMT

The effect of coil length from T to PMT was studied in the range  $10 - 250 \text{ cm}$ . The maximum CL signal was obtained on using  $50 \text{ cm}$  which was the suitable coil length for this study. Figure 7 shows the effect of coil length on the CL intensity.

##### Effect of some micellar solutions

The effect of some organized systems, including neutral surfactants (Triton X-100), cationic surfactants (cetyltrimethylammonium bromide, and cetylpyridinium chloride), and anionic surfactants (sodium dodecyl sulfate) was

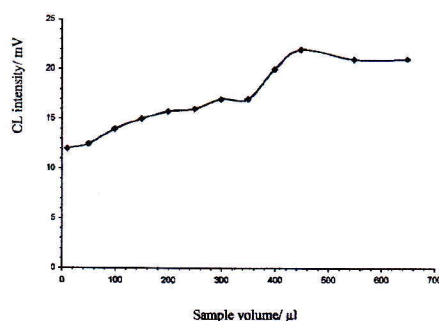


Fig. 6 Effect of sample volume on CL intensity of pioglitazone HCl. Injected drug solution,  $1.0 \mu\text{g ml}^{-1}$ ;  $[\text{Ce(IV)}] = 5 \times 10^{-4} \text{ M}$ ;  $[\text{Na}_2\text{SO}_3] = 1 \times 10^{-3} \text{ M}$ .

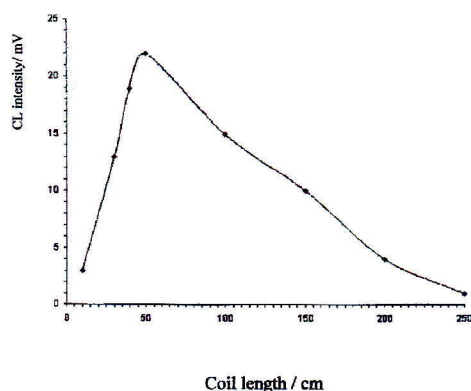
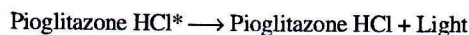
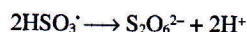


Fig. 7 Effect of coil length on CL intensity of pioglitazone HCl. Injected drug solution,  $1.0 \mu\text{g ml}^{-1}$ ;  $[\text{Ce(IV)}] = 5 \times 10^{-4} \text{ M}$ ;  $[\text{Na}_2\text{SO}_3] = 1 \times 10^{-3} \text{ M}$ ; loop size,  $450 \mu\text{l}$ .

investigated. The CL signals were disappeared or decreased, so they can not be considered as valuable parameters in this study.

#### CL mechanism

Pioglitazone HCl exhibits a native fluorescence in acidic solutions at 502 nm after excitation at 238 nm; therefore, it can be excited by energy transfer. Thus, the possible CL mechanism is:



Sulfite ion acts as the reductant; the energy from the chemical reaction is released as chemiexcites of sulfur dioxide, which emits radiation at wavelengths  $>300 \text{ nm}$ .<sup>26</sup> Pioglitazone HCl increases the weak radiation emitted during the CL oxidation of sulfite by  $\text{Ce(IV)}$  in sulfuric acid medium, so it acts as a sensitizer. This mechanism is similar to others reported previously.<sup>27-29</sup>

#### Determination of pioglitazone HCl

Under the described experimental conditions, a series of

Table 2 Determination of pioglitazone HCl in spiked serum and urine

Serum		Urine	
Concentration taken/ $\mu\text{g ml}^{-1}$	Found, %	Concentration taken/ $\mu\text{g ml}^{-1}$	Found, %
0.1	98.8	0.1	102.0
0.3	100.7	0.5	100.0
0.5	101.5	1.0	101.2
1.0	98.4	1.5	99.1
1.5	100.3	2.0	100.1
Mean $\pm$ SD	$99.9 \pm 1.30$	Mean $\pm$ SD	$100.5 \pm 1.13$

standard solutions of pioglitazone HCl were pumped, each as triplicate, to test the linearity of the calibration graph. A plot of the CL intensity vs. concentration of pioglitazone HCl was found to be linear over the range of  $0.05 - 3.0 \mu\text{g ml}^{-1}$ . The LOD was  $0.01 \mu\text{g ml}^{-1}$  ( $S/N = 2$ ) and a LOQ was  $0.05 \mu\text{g ml}^{-1}$  (which is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental condition). Linear regression analysis of the results gave the following equation:

$$I = 0.704 + 15.74C \quad (r = 0.9999, n = 9)$$

where  $I$  is the CL intensity in mV and  $C$  the concentration in  $\mu\text{g ml}^{-1}$ . The standard deviation of intercept  $\delta_a$  is 0.042 and the standard deviation of slope  $\delta_b$  is 0.026. The accuracy of the proposed method was checked by analyzing pioglitazone HCl in pure form. The % recoveries of the studied drug compared with those obtained by the spectrophotometric method<sup>20</sup> are given in Table 1.

#### Application of the method

**Analysis of pharmaceutical preparations.** In order to evaluate the analytical usefulness of the chemiluminescent method, we determined pioglitazone HCl in its pharmaceutical preparations. The results in Table 1 were in accordance with those obtained by the published spectrophotometric method.<sup>20</sup> Statistical analysis of the results by using the student's  $t$ -test<sup>30</sup> and the variance ratio  $F$ -test<sup>30</sup> showed no significant difference between the two methods as regards accuracy and precision (Table 1).

**Analysis of spiked biological fluids.** The high sensitivity attained by the proposed method allows the determination of pioglitazone HCl in biological fluids. Pioglitazone HCl is rapidly absorbed after oral administration. Peak plasma concentrations are obtained within 2 h and bioavailability exceeds 80%. Pioglitazone HCl is more than 99% bound to plasma proteins. It is extensively metabolized by cytochrome P450 isoenzymes CYP3A4 and CYP2C9 to both active and inactive metabolites. It is excreted in urine and faeces and has a plasma half-life of up to 7 h. The active metabolites have a half-life of up to 24 h.<sup>31</sup>

As a consequence, the proposed method appears to be convenient for the therapeutic drug monitoring in urine and serum. In addition, the combination of CL technique with flow injection, requiring smaller volumes of samples, may be valuable for routine drug screening of patients under treatment. The extraction procedure for biological fluids was performed by using dichloromethane, as reported by Kolte *et al.*<sup>32</sup> The CL-concentration plot is rectilinear over the concentration range  $0.1 - 1.5$  and  $0.1 - 2.0 \mu\text{g ml}^{-1}$  for urine and serum, respectively. Regression analyses of the results gave the following equations:



Table 3 Comparison of the analytical figures of merit of the proposed FI-CL method with earlier reported methods for the determination of pioglitazone HCl

Technique	Linear range/ $\mu\text{g ml}^{-1}$	Correlation coefficient, $r$	LOD $/\mu\text{g ml}^{-1}$	LOQ $/\mu\text{g ml}^{-1}$	Ref.
HPLC-UV at 269 nm					
C18 column, methanol/acetonitrile (pH 2.6)	0.05 – 0.2	0.9987	0.025	0.050	4
C18 column, H <sub>2</sub> O/acetonitrile/acetic acid (pH 5.5)	10 – 1000	—	0.0002	10	5
C8 column, acetonitrile containing acetic acid (pH 5.5)	10 to 2	—	—	0.02	6
HPLC-UV at 229 nm					
Acetonitrile containing acetic acid (pH 6.0)	0.025 – 20	—	0.010	0.025	7
HPLC-UV at 260 nm					
Formic acid/acetonitrile/H <sub>2</sub> O/methanol (pH 3.0)	0.1 – 100	—	—	0.1	8
HPLC-MS					
C18 column, ammonium acetate/acetonitrile	0.009 – 1.350	—	—	0.009	9
C18 column, acetonitrile/water/ammonium acetate	0.0005 – 2	0.998759	—	0.0005	10
C8 column, ammonium formate (pH 3.0)/methanol	—	—	0.010	—	11
HPTLC-UV at 266 nm					
1,4-Dioxane/phosphate buffer (pH 4.4)	40 – 240	0.9957	—	40	12
MEK	0.35 – 8.75	0.998	0.29	0.74	15
CE	0.001 – 0.0024	0.999	—	0.001	16
Potentiometric	2.5 – 3900	0.998	1.5716	2.3574	18
UV-second-derivative spectrophotometry	8 – 40	0.9984	—	8	20
UV-spectrophotometry	5 – 30	—	—	5	21
Proposed FI-CL method	0.05 – 3	0.9999	0.01	0.05	The proposed method

For urine:  $I = 0.29 + 4.85C$  ( $r = 0.9999$ ,  $n = 6$ )

For serum:  $I = 0.22 + 3.84C$  ( $r = 0.9997$ ,  $n = 5$ )

Table 2 shows the results for analyses of urine and serum samples. The intra-day precision was evaluated through triplicate analysis of a sample containing  $1.0 \mu\text{g ml}^{-1}$  of serum and urine. The mean % recoveries were  $99.0 \pm 1.38$  for serum and  $99.5 \pm 1.10$  for urine.

The inter-day precision was evaluated through triplicate analysis of a sample containing  $1.0 \mu\text{g ml}^{-1}$  of serum and urine on three consecutive days: the mean % recoveries were  $99.1 \pm 1.59$  for serum and  $99.7 \pm 1.30$  for urine.

#### Validity of the proposed method

**Linearity.** The proposed method was tested for linearity. The regression plot showed a linear dependence of CL intensity on pioglitazone HCl concentrations over the calibration range ( $0.05 - 3.0 \mu\text{g ml}^{-1}$ ). The LOD, LOQ as well as the slope and intercept were also clarified. Validation of the method was evaluated by statistical analysis of the regression line regarding  $\delta_a$  and  $\delta_b$ .

**Precision and accuracy of the method.** The intra-day precision was evaluated through five replicate analyses of a sample containing  $0.5 \mu\text{g ml}^{-1}$  of pioglitazone HCl. The mean % recovery was  $99.9 \pm 0.40$ . The inter-day precision was determined by triplicate analysis of a sample containing  $0.5 \mu\text{g ml}^{-1}$  on three consecutive days. The mean % recovery was  $99.7 \pm 0.64$ . The reproducibility was investigated using  $0.5 \mu\text{g ml}^{-1}$  ( $n = 15$ ) and the RSD % < 2, which illustrates that the results were highly reproducible.

The accuracy of the proposed method was evaluated by analyzing standard solution of the studied drug. The % found values of the studied drug compared with those obtained by the spectrophotometric method<sup>20</sup> are given in Table 1. Statistical analyses<sup>30</sup> of the results, obtained by the proposed and the published method<sup>20</sup> using the student's  $t$ -test and variance ratio  $F$ -test, showed no significant difference between the

performance of the two methods regarding the accuracy and precision, respectively.

#### Comparison of the proposed and the reported methods

Table 3 shows the results of comparison of the proposed method with other reported methods. All methods are equally accurate and precise. However, HPLC methods are generally tedious procedures and require special skills and sophisticated instrumentation. UV spectrophotometry has low sensitivity and selectivity. However, the proposed FI-CL procedure is highly sensitive, selective and offers the advantages of low-cost instrumentation. Also, it has reasonable determination range, short analysis time and significantly increased sample analyses rate.

#### Conclusion

The proposed FI-CL method is simple, accurate and precise. It has been used for the sensitive determination of pioglitazone-HCl in pharmaceutical preparations and biological fluids. It requires no sample pretreatment and solutions can be analyzed at a rate of 50 samples  $\text{h}^{-1}$ ; a simple extraction procedure has been used for urine and serum samples.

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