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DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PIOGLITAZONE HCL FROM TWO DIFFERENT MARKETED BRANDS

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ABSTRACT

Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the quantitative estimation of Pioglitazone, in bulk drug and pharmaceutical dosage forms. Methods were based on the formation of Pale yellow coloured and green coloured chromo gens, which were measured 267 nm and 297 nm, respectively. The results obtained with the proposed methods are in good agreement with the labeled amounts when tablet dosage forms were analyzed. For the first method, UV-spectrophotometry, standard solutions were measured at 267 nm. The first method was linear from 2.5-20 mg/mL. The second method was based on the formation of an ion association complex with Methyl orange (MO) and Bromocresol Green (BCG).The assay was found to be linear over the concentration range of 2.5-20 µg/mL. The wavelengths were selected dependent on the maximum obtained values. The formation of ion-pairs are formed between secondary amino group of PIO and MO, BCG reagents via the protonated nitrogen atom. The proposed methods were validated according to the ICH guidelines (1996) with respect to specificity, linearity, accuracy, precise and robustness. The results demonstrated that the procedure is accurate, precise, specific and reproducible (percent relative standard deviation <2%), while being simple and less time consuming. The two methods have been successfully applied to the assay of Pioglitazone.

Key words: Antidiabetic, PIO, BCG, MO, UV-spectroscopy, Validation, Ion pair

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INTRODUCTION

Pioglitazone(PIO)[(_)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4 thiazolidinedione monohydrochloride (Merck Index 2001) (Figure 1).

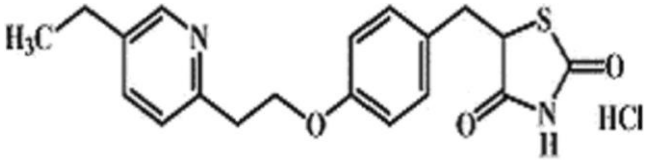

Figure 1. Molecular Structure of Pioglitazone HCl

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action. The drug PIO selectively stimulates nuclear receptor peroxisone proliferator-activated receptor gamma (PPARc). It act as or it modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the lipidic muscular tissues and in the liver. As a result, PIO reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; and reduces the quantity of glucose, insulin, and glycated hemoglobin in the bloodstream. Although not clinically significant, PIO decreases the level of triglycerides and increases that of high-density lipoproteins (HDL) without changing low-density lipoproteins (LDL) and total cholesterol in patients with disorders of the lipid metabolism, although statins are the drug of choice for this condition[1]. Few methods have been reported for the determination of PIO in pharmaceutical dosage forms. The HPLC and micellar electro-kinetic chromatographic (MEKC) methods have been studied for the determination of PIO in bulk and pharmaceutical formulations[2]. The detection of PIO and glimepiride were determined in tablet simultaneously by HPLC[3]. Gumieniczek, Hopkala and Berecka[4] have reported a reversed-phase thin-layer chromatography and densitometry for the determination of PIO in tablets and studied HPLC and thin-layer chromatography for the determination of PIO in bulk. Simultaneous HPLC method has been developed for the determination of PIO and metformin in the pharmaceutical dosage form[5-6] and HPLC for the determination of PIO pharmaceutical formulations and human plasma was reported. Reversed-phase HPLC method has been used for the quantitative determination of PIO in tablets. The objective of the present study was to develop simple, precise, accurate, and validated economic analytical methods for the determination of Pioglitazone preparations.

MATERIAL AND METHODS

Materials

Pioglitazone and its tablets PIOMED and PEPAR were kindly supplied from IPCA and Glen mark Pharmaceuticals .Bromocresol green (BCG) and Methyl orange (MO) were purchased from Qualigens fine chemicals (Mahape Navi-Mumbai).

Apparatus

Solvents and other chemicals were of analytical grade. Perkin Elmer Model –Lamda 25. UV-VIS spectrophotometer with 1 cm quartz cells was used. UV-Visible spectra were automatically obtained by Perkin Elmer's UV-Lamda 25 system software. Speed was 2400 nm, scan range 250–450 nm, slit width 2nm.

Solutions and Methods

General Procedure

Ultraviolet (UV) Spectrophotometric Method. A quantity of 100 mg PIO was accurately weighed and dissolved in 100.0mL ethanol and rest volume with solvent (Methanol: Water). Standard solutions were obtained by diluting the stock solution for the preparation of calibration curves in the concentration range of 2.5–20.0 µg/mL. The absorbance was measured at 267 nm.

Colorimetric Method (Ion Pair Complex). Standard stock solution of PIO, 1000 µg/mL_1, was prepared by dissolving 50 mg of the drug in 5.0mL methanol and diluted to 45mL with solvent (Methanol: Water). Working standard solution was prepared as required by suitable dilution of the stock solution with solvent. Solution of BCG, MO 0.04% (w=v) were prepared in chloroform. The acid dyes solutions were prepared fresh daily. The PIO-BCG, PIO-MO ion pair complexes were adjusted to 10 ml with solvent (Figure 2). The absorbance was measured at 297 and 267 nm for PIO-BCG, MO-PIO complexes, respectively, against a reagent blank similarly prepared.

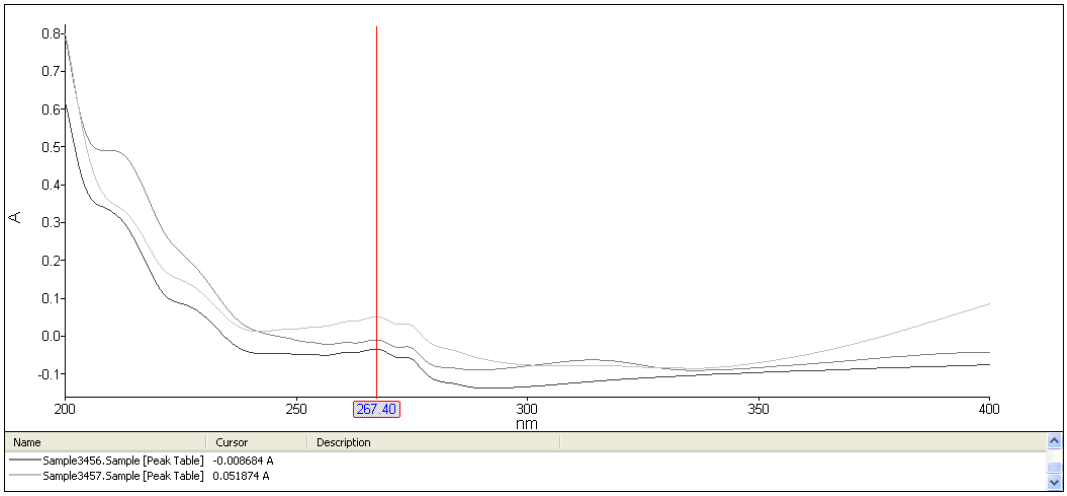

Figure 2. Spectrum of Pioglitazone, Methyl Orange and Bromocresol Green

Preparation of Tablet Sample Solution

Twenty tablets were weighed and powdered, and then a quantity of the powder equivalent to 10 mg of PIO was transferred into a 100mL volumetric flask containing 50.0mL methanol and volume make up with solvent. For the UV spectrophotometric method, a 1.0mL aliquot of the resulting solution was transferred to a 10mL volumetric flask and the volume was adjusted with solvent. For ion pair (ion association complex) method. The assay of PIO content were completed as described in the section on general procedure.

Analytical Method Validation

Specificity

PIO solutions PIO solutions (10 µg/mL) were prepared, separately, in both the selected media along with and without common excipients. All the solutions were scanned from 800 to 200nm and checked for any change in the absorbance at respective wavelengths. In a separate study, drug concentration 10 µg/mL was prepared independently from pure drug stock in the selected media and analyzed (n¼6)

Linearity

The calibration curves were constructed by plotting concentration versus absorbance, using linear regression analysis. (Table 1)

Table 1: Linearity study of pioglitazone

Sr No.	Concentration of Pioglitazone [µg/ml]	Absorbance Mean ± S.D. [n = 5]	R^2
1	2.5	0.055	
2	5	0.105	
3	10	0.203	0.999
4	15	0.286	
5	20	0.368	

SD-Standard Deviation, R^2-Correlation Coefficient

Accuracy

As a part of determining accuracy of the proposed methods, different levels of drug concentrations (low concentration, medium concentration, and high concentration in both media) were prepared from independent stock solution for both the formulation and analyzed. Accuracy was assessed as the Standard deviation.(Table 2A and 2B).

Precision

Repeatability was determined by using different levels of drug concentrations prepared from independent stock solution and analyzed .Inter-day and intra-day variations were taken to

Table 2(A): E (1%, 1cm) of pioglitazone at 267.0 nm (methyl orange)

Sr No.	Concentration	Absorbance	E(1%,1cm)
1	10	0.203	260.0
2	10	0.205	256.25
3	10	0.206	258.75
4	10	0.205	261.25
5	10	0.206	256.25
AVG		259.500	
SD		1.27475	
% R.S.D.		0.49	

Table 2(B): E (1%, 1cm) of pioglitazone at 297.0 nm (bromocresol green)

Sr No.	Concentration	Absorbance	E(1%,1cm)
1	10	0.064	80.0
2	10	0.064	78.75
3	10	0.064	81.25
4	10	0.064	82.50
5	10	0.064	77.50
Mean ± S.D.		0.0644 ± 0.00114	
% R.S.D.		1.7704	

Table 3(A): Precision and accuracy of proposed methods (Intraday=5)

Method	Absorbance	%RSD
Intra-day		
1	0.209	
2	0.207	**0.55**
3	0.206	
4	0.208	
5	0.207	

RSD-Relative Standard Deviation

Table 3(B): Precision and accuracy of proposed methods (Interday=6)

Method	Absorbance	%RSD
Inter-day		
1	0.202	
2	0.207	
3	0.205	
4	0.209	1.44
5	0.202	

RSD-Relative Standard Deviation

determine intermediate precision of the proposed methods. Different levels of drug concentrations in triplicates for drug were prepared three different times in a day and studied for

intra-day variation. The same method was followed for three different days to study inter-day variation (Table 3 (a) and (b)).

Recovery

The % recovery of the added pure drug was calculated as, %recovery¼ [(.Ct_Cs)=Ca]_100, where Ct is the total drug concentration measured after standard addition; Cs, drug concentration in the formulation sample; and Ca, drug concentration added to formulation.(Table 4) Assay results for the determination of PIO in commercial tablets by the proposed methods (PIOMED and PEPAR 15 mg) (Table 5(a) and (b)).

Table 5 (A): Assay of pioglitazone marketed preparation –For Piomed (15 mg)

STANDARD	0.208	10 PPM
Sr. No.	**Abs.**	**% Assay**
1	0.206	101.43
2	0.205	101.84
3	0.208	101.84
4	0.207	101.02
5	0.208	101.43
	Avg	101.51
	SD	0.3421
	RSD	0.337091

Table 5 (B): Assay of pioglitazone marketed preparation – For Pepar (15mg)

STANDARD	0.208	10 PPM
Sr. No.	**Abs.**	**% Assay**
1	0.206	99.67
2	0.207	98.84
3	0.208	98.84
4	0.207	99.67
5	0.207	98.84
	Avg	99.17
	SD	0.453043
	RSD	0.456816

Robustness

For the evaluation of the methods robustness, some parameters were interchanged: reagent concentration and kmax and shaking time. The capacity remained unaffected by small deliberate variations. (Table 6)

RESULTS AND DISCUSSION

Absorption Spectra

UV absorption spectrum of PIO showed a maximum absorbance at 267 nm (Figure 3). These wavelengths were selected dependent on the maximum obtained values. This work is undertaken in the view that ion-pairs are formed between secondary amino group of PIO and BCG and MO reagents via the protonated nitrogen atom. On adding the basic drug solution, stable yellow ion-pairs are formed. The maximum absorption wavelengths were measured at 297 nm and 267 nm for PIO-BCG, PIO-MO respectively .Optimum Reaction Conditions for Complex Formation.

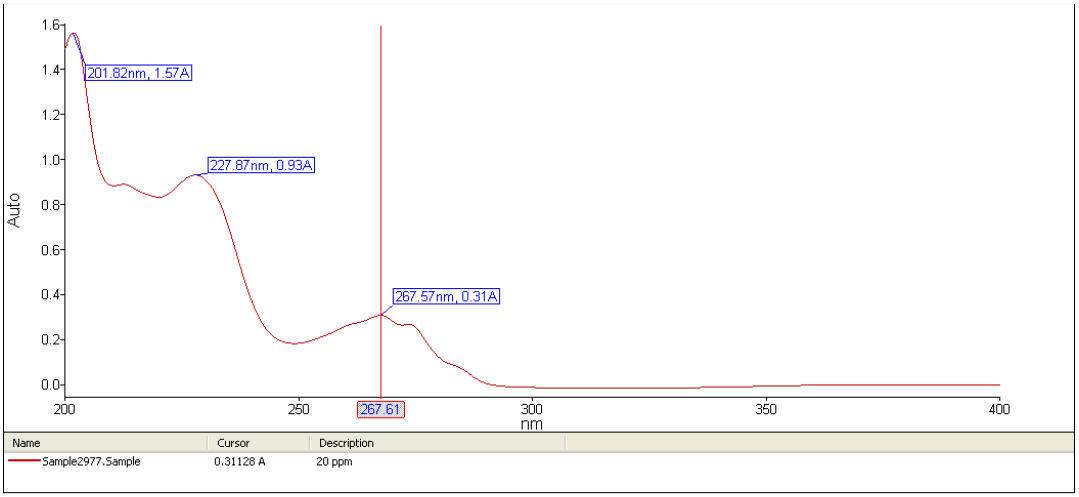

Figure 3. Spectrum of Pioglitazone Plain

The optimization of the methods was observed carefully studied to achieve complete reaction formation, highest sensitivity, and maximum absorbance. Reaction conditions of the ion-pair complex were found through preliminary experiments studying conditions such as, type of organic solvent, volumes of the dye. Containing basic cationic nitrogen, PIO reacts (Figure 1). Chemical structures of acid dyes are in (Figure 4) with BCG, MO and acid dyes, to form a colored product. Therefore, yellow color is produced. This is due to conversion of the dye into an open quinonoidal anionic derivative, which forms an ion-pair with PIO. In the proposed method, the reaction is carried out in organic solvent (chloroform) and, hence, is pH-independent; as a result, the ionization of the dye depends on the concentration of the basic drugs[7].

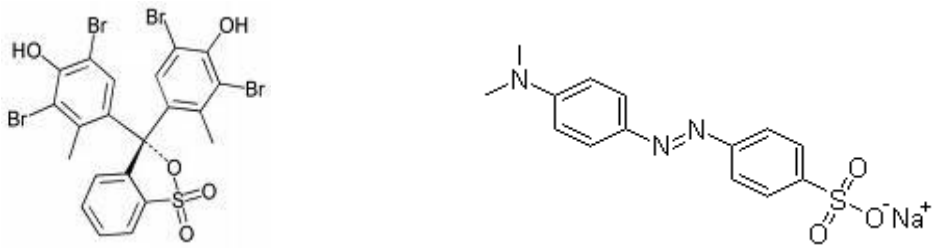

Figure 4. Bromocresol Green (BCG) and Methyl Orange (MO)

Effect of Time and Temperature

Complete color intensity was attained after 2 min of mixing for all complexes. The optimum reaction time was investigated by following the color development at ambient temperature. The intensity of ion-pairs extraction was found to be stable at room temperature. (25±2˚C)

Effect of Reagents Concentration

The optimum conditions for reagents and PIO methods were established by varying the concentration of reagent at a time and keeping the fixed drug concentration and observing the effect produced on the colored species.

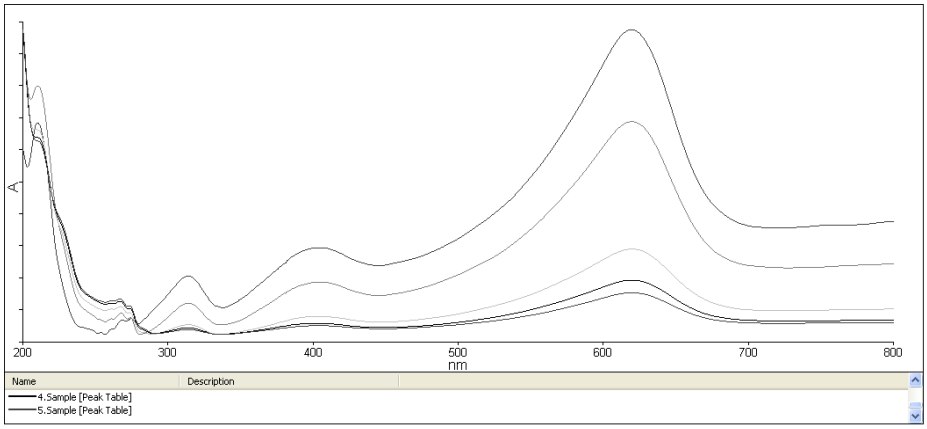

Figure 5. Spectrum of BCG with varying concentration of Drug Pioglitazone

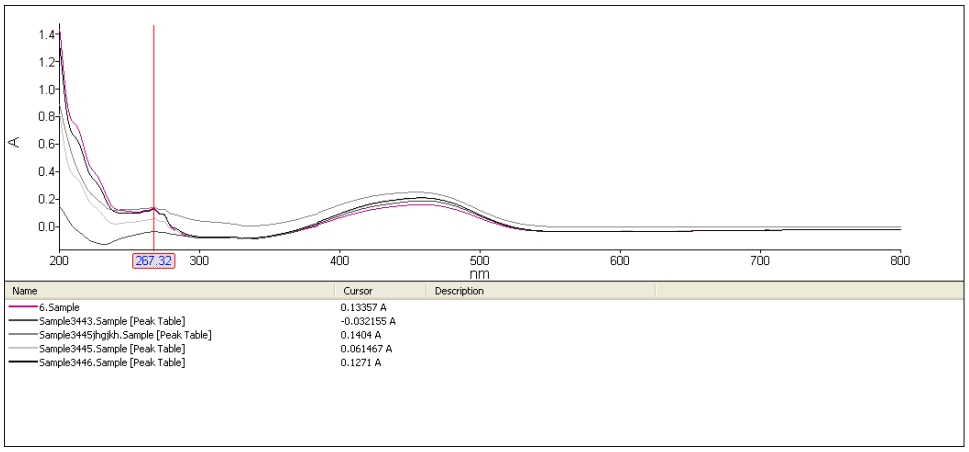

Figure 6. Spectrum of Methyl Orange with varying concentration of drug Pioglitazone

The effect of the concentration of reagents (BCG and MO) on the color intensity was studied using different volumes of 0.1 and 0.05% solutions. For BCG and MO results were obtained in a 0.05% (w/v) solution. (Figure 5, 6)

Method Validation

Specificity

The absorption spectrum of the pure drug sample matched the marketed formulation sample in both the selected media. The excipients present in all tablets did not interfere with the drug. (Table 7)

Table 7: Specificity

Sr No.	Condition (24 hr)	Wt taken	Abs	% of Drug
1	0.1 N HCl	43.4	0.119	82.07
2	0.1 N NaOH	43.5	0.181	74.09
3	3% H_2O_2	43.6	0.212	85.53
4	Heat	43.7	0.211	88.21
5	Normal	43.5	0.202	84.05

Linearity

Therefore, the proposed methods are specific and selective for the drug. Calibration curves of PIO were linear over the concentration range of 2.5–20.0 µg/mL for the UV method, 2.0–12.0 µg/mL for the derivative spectrophotometric method, 2.5-20 µg/mL for BCG and same for the MO. (Figure 7)

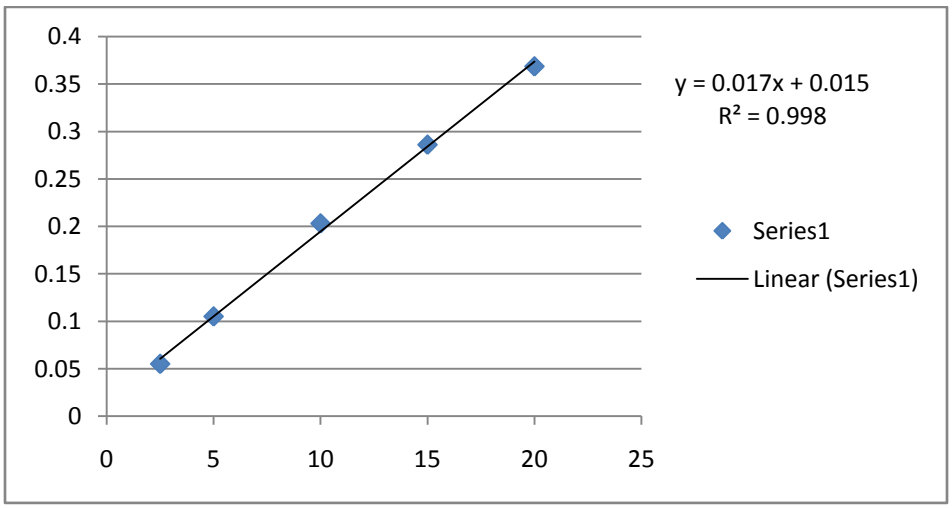

Figure 7. Calibration curve of Pioglitazone HCl

Precision and Accuracy

Table 3 (a) and (b) show the obtained values for precision (RSD<2 % for the UV method, and RSD<2 for the ion pair method). The repeatability and reproducibility of the three methods are fairly good as indicated by the low values of RSD%.

Recovery

The recovery study was performed by adding known amounts of the compounds studied to a known concentration of the commercial pharmaceutical tablets (standard addition method). For the UV spectrophotometric method, the mean recoveries for PIO at 80,100,120 PPM (total concentration) were 98.91%, 99.13% and 101.02%, respectively. For derivative spectrophotometric method, the mean recoveries for PIO at 2.0, 8.0, and 12.0µg/mL were 101.25%, 99.0% and 100.8%, respectively. For the ion pair method, the mean recoveries for PIO at 80.0, 100.0, and 120.0µg/mL were 101.2%, 99%, and 100.80%, respectively (Table 4).

Table 4: Recovery study of pioglitazone tablets

	Recovery (%)	**RSD (%)**
UV spectroscopy(for **Piomed)**80 ppm	101.39	**0.2646**
100ppm	99.57	
120 ppm	100.86	
For **Pepar**(80ppm)	99.23	
100 ppm	99.52	**1.005**
120 ppm	100.96	

RSD-Relative Standard Deviation

Robustness

The proposed methods conditions are robust. The robustness of the spectrophotometric method was determined by analysis of samples under (Table 6). Analytical parameters for the determination of PIO using the proposed methods.

Table 6: Robustness study

ROBUSTNESS			**ROBUSTNESS**	
Sr. No.	**Wavelength**	**Abs.**	**Wavelength**	**Abs.**
1	269 nm	0.209	265 nm	0.205
2	269 nm	0.206	265 nm	0.204
3	269 nm	0.207	265 nm	0.203
4	269 nm	0.208	265 nm	0.205
5	269 nm	0.207	265 nm	0.204
6	269 nm	0.208	265 nm	0.205
	Avg.	0.209	Avg.	0.205
	SD	**0.0011**	**SD**	**0.000837**
	RSD	**0.55**	**RSD**	**0.41**

Table 8: For summary report

PARAMETER	ACCEPTANCE CRITERIA	RESULTS
LINEARITY CORRELATION COEFFICIENT	Correlation coefficient was 0.999	Graph should be Linear r= ≥ 0.999
PRECISION METHOD PRECISION	The RSD of 6 replicates was within the limit; 0.1089	% RSD : NMT 2.0.
ROBUSTNESS	Meets the acceptance criteria.	NMT 1.0% of each other.
ASSAY FOR BOTH BRAND	Meets the acceptance criteria	Within 95-105%
ACCURACY	Meets the acceptance criteria.	RSD : NMT ≤ 2.0.

CONCLUSIONS

The proposed methods have been proved to be simple, precise, rapid and reliable. The developed method reported here in was validated by evaluation of the validation parameters as described in the ICH-Q2B guideline for specificity, linearity are within- and between day precision, and accuracy of the proposed technique which were obtained during the validation studies. The method is very simple, no pH-adjustment and no expensive instrumentation. The UV, derivative spectrophotometric, and ion pair methods are more specific than the other methods[8-11]. The derivative spectrophotometric methods have the advantages of lower cost, rapid results, and environmentally protective. Unlike the gas chromatographic and HPLC procedures, the instrument is simple and inexpensive. The method can be successfully employed for PIO quantification in all types of pharmaceutical preparations and liquid samples, such as urine and plasma.

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