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Spectrophotometric assay of pioglitazone hydrochloride using permanganate in acidic and basic media

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A B S T R A C T

Pioglitazone hydrochloride (PGH) is an oral anti-hyperglycemic agent used in the treatment of type-2 diabetes mellitus. Potassium permanganate was found to oxidize PGH both in acidic and basic conditions, based on which two simple and sensitive methods were developed for its determination in bulk sample and tablets, and validated. In the first method (indirect method), PGH was reacted with a measured excess of standard permanganate in H_2SO_4 medium, and the residual oxidant was determined by measuring its absorbance at 550 nm. The second method (Direct method) entails treating PGH with permanganate in NaOH medium, followed by the measurement of the resulting bluish-green manganite at 610 nm. Experimental variables affecting the reactions were studied and optimized. Under optimum conditions, linear relationships with good correlation coefficients were found between absorbance and concentration in the ranges, 1.25 – 25 μg mL^{-1} (Indirect method) and 1-12 μg mL^{-1} (Direct method) with respective molar absorptivity values of 1.10×10^4 and 2.77×10^4 l mol^{-1} cm^{-1}. The limits of detection (LOD) and quantification (LOQ) were 0.36 and 1.08 (Indirect method) and 0.23 and 0.69 μg mL^{-1} (Direct method). Intra-day and inter-day precisions were satisfactory, with %RSD values of ≤2.11, and the respective accuracies were excellent with %RE values of ≤2. The methods were also validated for robustness, ruggedness and selectivity. The methods were applied to the determination of PGH in its tablets with good accuracy and precision, and no interference from the tablet additives was encountered. The results were also compared with those obtained by a reference method.

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1. Introduction

Pioglitazone hydrochloride (PGH), chemically known as 5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-thiazolidinedione monohydrochloride (Fig. 1),[1] is an oral anti-hyperglycemic agent.[2]It addresses the main pathophysiological defects, i.e., insulin resistance, and hence used alone or in combination with insulin, metformin or sulphonyl ureas (glipizide and glibenclamide), as an agent to treat diabetes.[3]

No monographs are available in any pharmacopeia for assay of this drug. Various analytical methods developed for its determination in pharmaceuticals and biological samples have recently been

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reviewed.[4] Various techniques such as uv-spectrophotometry,[5-23] potentiometry,[224-26] voltammetry,[27-28] high-performance liquid chromatography,[29-39] ultra-performance liquid chromatography,[40] high-performance thin layer chromatography[41-46] and capillary electrophoresis[47] have been reported for the determination of PGH in bulk sampleand tablets.

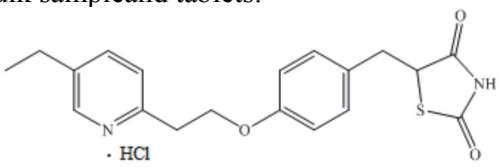


Fig. 1. Structure of PGH

Visible spectrophotometry is still the most sought-after technique in industrial quality control and research laboratories because of its speed, cost-effectiveness, ease of performance, and fair selectivity and sensitivity. Despite these advantages, technique has been scantily applied for the determination of PGH, with only two reports being found in the open literature[9, 10]. PGH is reported to form ion-pair complexes with acidic dyes: methyl orange and bromocresol green, in chloroform and these complexes were measured at 267 and 297 nm, respectively, allowing its determination in 2.5 – 20 μg mL^{-1} range.[9] In a similar method[10] using bromocresol green as ion-pair agent, the coloured species formed in phthalate buffer of pH 2.4 was extracted into chloroform and absorbance measured at 419 nm. Beer's law was obeyed over 2.5 -14 μg mL^{-1} concentration range and the method was applied to tablets. In the first case,[9] absorbance is measured in the uv region where the interference from co-formulated substances is expected to be high and the extractive method[9] has several disadvantages such as need for pH adjustment, liquid-liquid extraction step and use of large quantities of organic solvents. Thus arises, need for simple, facile and reliable visible spectrophotometric methods for the determination of PGH in pharmaceuticals.

In the presence of reducing agent, potassium permanganate gets reduced to different oxidation states in both acidic and basic media. In acidic solution, manganese(VII) is reduced to manganese(II), whereas in basic medium, it gets reduced to manganese(VI) as shown below:[48]

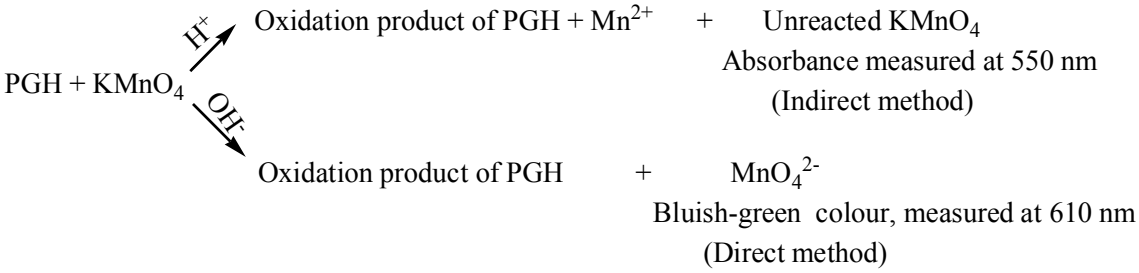
$$MnO_4^- + 8H^+ + 5e^- = Mn^{2+} + 4H_2O$$
$$MnO_4^- + e^- = MnO_4^{2-}$$

The innate intense purple color of permanganate solution which absorbs in the vicinity of 550 nm and the bluish-green color of manganite ion[48] with a λ_{max} at 610 nm, the reduced form of permanganate in alkaline medium have been exploited for the spectrophotometric determination of many pharmaceutical compounds.[49-60] In spite of its extensive application in pharmaceutical analysis, permanganate, as per the literature, has not been used for the determination of PGH. In this work, permanganate was used as an oxidimetric agent for developing two spectrophotometric methods. In the Indirect method, the residual permanganate was measured at 550 nm, after allowing the reaction between PGH and known amount of oxidant in H_2SO_4 medium. Whereas in the direct method, the bluish-green color of manganite, the product of reaction between drug and permanganate in alkaline medium, was measured at 610 nm, which served as the basis of the Direct method. The methods were found to be much simpler and more sensitive than the existing spectrophotometric methods.

2. Results and discussion

The proposed methods are based on the redox reaction between permanganate and PGH in acid (Indirect method) or in basic (Direct method) medium. In Indirect method, a known excess of standard $KMnO_4$ was added to PGH in acid medium followed by the determination of the residual oxidant by measuring its absorbance at 550 nm. The decrease in absorbance at 550 nm with respect to water blank was taken as the measure of PGH concentration. In Direct method, K_2MnO_4 resulting from the

reduction of $KMnO_4$ by PGH in alkaline medium was measured at 610 nm and related to PGH concentration. The possible reaction pathways and basis of assays are given in Scheme 1.



Scheme 1. Reaction pathways and basis of determination

1.1. Optimization of experimental conditions

1.1.1. Indirect Method

Preliminary experiments were performed to determine the permanganate concentration which would give a reasonable maximum absorbance at 550 nm in H_2SO_4 medium; and this was found to be 60 μg mL^{-1}. Hence, different concentrations of PGH were reacted with 1 mL of 600 μg mL^{-1} $KMnO_4$ in acid medium, and after the elapsed contact time, the absorbance of the residual permanganate was measured and related to PGH concentration. When a fixed concentration of $KMnO_4$ (60 μg mL^{-1}) was reacted with varying concentrations of PGH, the former was consumed in proportion to PGH concentration and there occurred a concomitant fall in the concentration of $KMnO_4$ as shown by the decreasing absorbance values at 550 nm with increase in the PGH concentration. This is depicted in **Fig. 2.** This facilitated the evaluation of the linear range over which the method could be applicable to the determination of PGH.

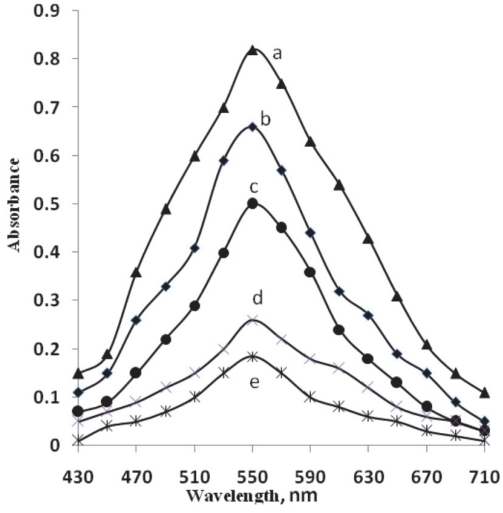


Fig. 2. Absorption spectra of $KMnO_4$ (60 μg mL^{-1}) after reacting with PGH (μg mL^{-1}):

a) 0.0, **b**) 5.0, **c**) 10.0, **d**) 17.5 and **e**) 20.0

Sulphuric acid is the most suitable acid, since it has no action upon permanganate in dilute solution. With hydrochloric acid, there is the likelihood of the reaction taking place and some permanganate may be consumed in the formation of chlorine.[61] Hence, the reaction of the oxidant with the drug was carried out in H_2SO_4 medium. Experiments were performed with 0.5-3.0 mL of 2 M H_2SO_4 and it was found that constant and reproducible absorbance readings were obtained in the range studied. Hence, 2 mL of 2M H_2SO_4 in a total volume of 10 mL was fixed as the optimum. The redox reaction with 10 μg mL^{-1} PGH was complete in 15 min, and the absorbance of residual oxidant remained constant for the next 45 min at room temperature (28±2 ^{O}C).

2.1.2. Direct method

This method is based on the reduction of permanganate to manganite by PGH in the presence of NaOH, the bluish-green colored chromogen[62] having the absorption maximum at 610 nm (**Fig. 3**). The formation of the colored product and the sensitivity of the reaction were found to be influenced by the alkali and permanganate concentrations. Maximum and constant absorbance readings were observed with 1 mL of 0.5M NaOH in a total volume of 10 mL (**Fig. 4a**). The reaction took 10 min for completion, and the bluish-green manganite color was stable for 40 min thereafter (**Fig 4b**). When a separate experiment was conducted to study the effect of permanganate concentration, it was found that maximum absorbance associated with a minimum blank reading was obtained when 1 mL of 0.1% $KMnO_4$ in a total volume of 10 mL was used. Higher concentrations of permanganate resulted in increased sensitivity, but the blank absorbance showed an increasing trend simultaneously.

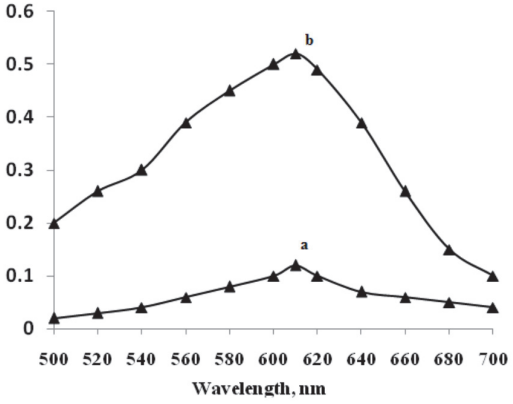
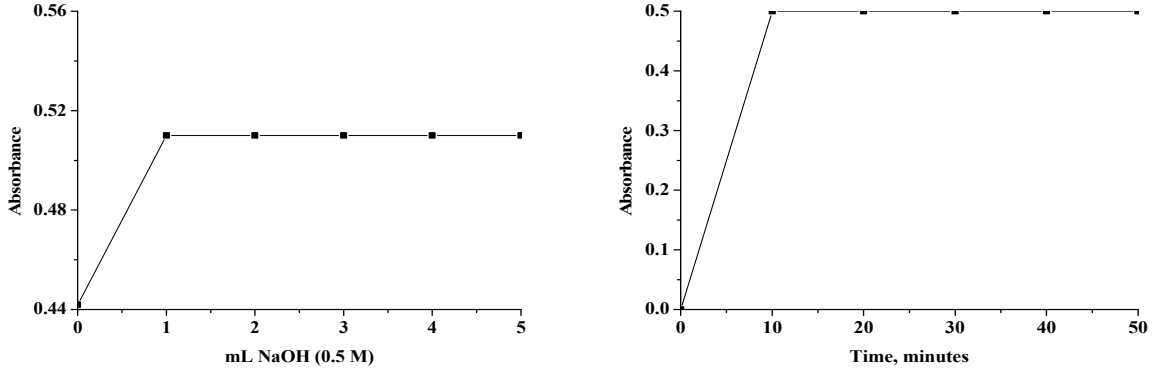


Fig. 3. Absorption spectra of: **a**) Blank, **b**) bluish-green color produced for 6 μg mL^{-1} PGH, in Direct method



(a) (b)

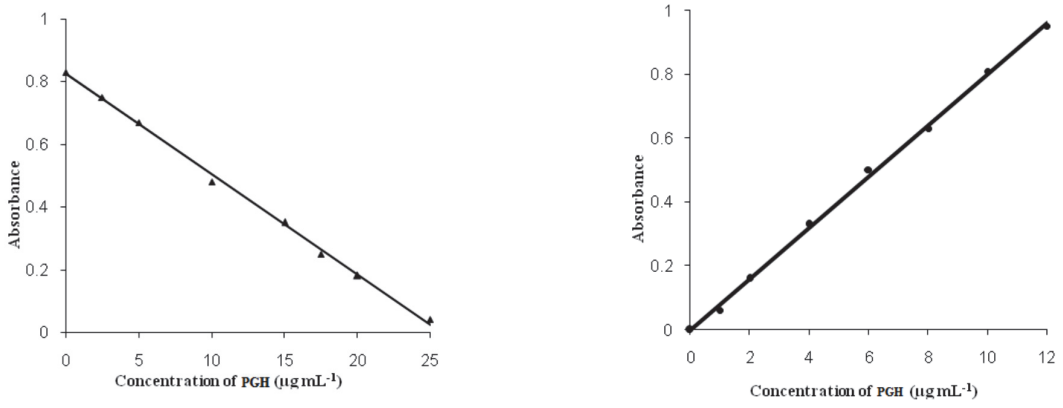
Fig. 4. Effects of: **a)** NaOH concentration, **b)** reaction time and stability of colored species (6 μg mL^{-1} PGH)

2.2. Method validation

2.2.1 Linearity, sensitivity, limits of detection and quantification

A linear correlation was found between absorbance at λmax and concentration of PGH (**Fig. 5**). The slope (m), intercept (b) and correlation coefficient (r) for each system were evaluated using the method of least squares. Optical characteristics such as Beer's law limits, molar absorptivity and

Sandell sensitivity values are presented in **Table 1**. The limits of detection (LOD) and quantitation (LOQ) are also calculated according to *ICH* guidelines[63] and these data are presented in **Table 1.**



(a) (b)

Fig. 5. Calibration curves: **a**) Indirect method and **b**) Direct method

Table 1. Sensitivity and regression parameters

Parameter	Indirect method	Direct method
λ_{max}, nm	550	610
Linear range, µg mL^{-1}	1.25 – 25.0	1.0-12.0
Molar absorptivity(ε), L mol^{-1} cm^{-1}	1.16×10^4	2.77×10^4
Sandell sensitivity*, µg cm^{-2}	0.0315	0.0128
Limit of detection (LOD), µg mL^{-1}	0.36	23
Limit of quantification (LOQ), µg mL^{-1}	1.08	0.69
Regression equation, y*		
Intercept (b)	0.821	0.0024
Slope (m)	-0.0318	0.0804
Standard deviation of b (S_b)	0.0017	0.0009
Standard deviation of m (S_m)	7.52×10^{-4}	1.53×10^{-3}
Correlation coefficient (r)	-0.9972	0.9991

*y=mx+b, where y is the absorbance, x concentration in µg mL^{-1}, b intercept, m slope.

2.2.2. Precision and accuracy

To check the precision and accuracy of the proposed methods, the assays described under "general procedures" were repeated seven times within the day (intra-day precision and accuracy) and five times on five different days (inter-day precision and accuracy). These assays were performed at three levels of analyte. The RSD values were ≤2.06% (intra-day) and ≤ 2.11% (inter-day) indicating high precision of the methods. %RE values of ≤ 2.0% demonstrate the fair accuracy of the proposed methods. The results of this study are summarized in **Table 2**.

Table 2. Results of accuracy and precision study

Method	PGH taken, µg mL^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
		PGH found, µg mL^{-1}	%RE	%RSD	PGH found, µg mL^{-1}	%RE	%RSD
Indirect	15	15.15	1.00	1.89	15.19	1.27	2.11
	20	20.19	0.95	1.45	20.21	1.05	1.35
	25	25.23	0.92	2.06	25.26	1.04	1.48
Direct	4	4.06	1.50	1.67	4.08	2.00	1.25
	6	6.10	1.67	1.48	6.11	1.83	0.95
	8	8.13	1.62	1.88	8.14	1.75	1.50

RE-Relative error, RSD-Relative standard deviation

2.2.3. Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent and reaction time and the effects of the changes were studied. To determine ruggedness, analyses were performed by three different analysts, and also with three different cuvettes by a single analyst. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness, was within the acceptable limits as shown in the **Table 3**, reflecting the robustness and ruggedness of the methods.

Table 3. Results of robustness and ruggedness study expressed as intermediate precision (%RSD)

Method	PGH taken, µg mL^{-1}	Robustness (%RSD)		Ruggedness (%RSD)	
		Conditions altered*			
		Reaction time* (min)	Volume of reagent** (mL)	Inter-analysts (n=4)	Inter-cuvettes (n = 4)
Indirect	15	0.89	0.98	0.57	1.34
	20	1.09	1.24	0.85	1.09
	25	0.86	0.69	0.36	0.58
Direct	4	1.12	0.63	0.89	1.05
	6	1.38	1.15	0.87	1.24
	8	1.88	0.92	1.07	1.56

*Reaction times were 13, 15 & 17 min in Indirect method and 8, 10 & 12 min in Direct method.
**In Indirect method, H_2SO_4 volumes were 1.8, 2.0 and 2.2 mL, and NaOH volumes in Direct method were 0.8, 1.0 and 1.2 mL.

2.2.4. Selectivity

There was hardly any change in the absorbance of permanganate when reacted with placebo blank (Indirect method) and no discernible colour developed in direct method. The percentage recoveries of PGH from synthetic mixture solution were 99.67±1.94 for Indirect method and 98.72±1.45 for Direct method. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of PGH.

2.2.5. Application to tablets

The proposed methods were applied to the quantification of PGH in commercial tablets. The results were compared with those of reference method.[5] The reference method involve analytical procedure for measurement of absorbance of PGH at 269 nm in 0.1 M HCl. of The accuracy and precision of the proposed methods were further evaluated by applying Student's t- test and variance ratio F- test, respectively. The t- and F-values at 95% confidence level did not exceed the tabulated values and this further confirms that there is no significant difference between the reference method and proposed methods with respect to accuracy and precision. The results of this study are presented in Table 4.

Table 4. Results of analysis of tablets by the proposed methods

Tablet brand name	Label claim, mg/tablet	Found* (Percent of label claim ±SD)		
		Reference method	Proposed methods	
			Indirect Method	Direct Method
Oglo-15	15	98.99±1.54	98.47±2.14 t= 0.44 F= 1.93	99.45±1.07 t= 0.55 F= 2.07
Neoglit-30	30	100.5±1.66	99.01±2.05 t= 1.29 F= 1.53	98.47±2.14 t= 1.7 F= 1.66

*Mean value of five determinations

2.2.6. Accuracy assessment by recovery study

Recovery test was performed by applying the standard-addition procedure. The test was done by spiking the pre-analyzed tablet powder with pure PGH at three different levels (50, 100 and 150%) of the content present in the tablet powder (taken) and the total was determined by the proposed methods.

Each test was repeated three times. The recovery percentage values ranged between 98.56 and 103.1% with standard deviation in the range 0.98-2.01% reflecting the high accuracy as well as selectivity of the methods. The results are shown in Table 5.

Table 5. Results of recovery study by standard addition method

Tablet studied	Indirect method				Direct method			
	PGH in tablet, μg mL^{-1}	Pure PGH added, μg mL^{-1}	Total PGH found, μg mL^{-1}	Pure PGH recovered (Percent±SD*)	PGH in tablet, μg mL^{-1}	Pure PGH added, μg mL^{-1}	Total PGH found, μg mL^{-1}	Pure PGH recovered (Percent±SD*)
Oglo-15	9.85	5.0	15.00	101.1±1.23	3.98	2.0	6.11	102.2±1.12
	9.85	10.0	20.25	102.0±1.05	3.98	4.0	8.18	102.5±0.98
	9.85	15.0	24.49	98.56±1.98	3.98	6.0	10.15	101.7±1.39
Neoglit-30	9.90	5.0	15.12	101.5±1.11	3.94	2.0	6.03	101.5±1.90
	9.90	10.0	20.39	102.5±1.78	3.94	4.0	8.18	103.1±1.40
	9.90	15.0	25.44	102.2±2.01	3.94	6.0	10.13	101.9±1.78

*Mean value of three measurements

3. Conclusion

Reaction of pioglitazone with permanganate in acid and basic media was successfully exploited for the spectrophotometric determination of drug. The reactions provide relatively simple and rapid means of assay of pivoglitazone and its tablet dosage form. The proposed methods are more sensitive than the presently available methods, and have wider linear dynamic ranges.

Acknowledgement

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5. Experimental

5.1. Apparatus

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped with 1-cm matched quartz cells was used for absorbance measurements.

5.2. Materials and reagents

All chemicals used were of analytical reagent grade and double distilled water was used throughout the study.

An approximately 0.01M solution of potassium permanganate was prepared by dissolving 0.395 g of the chemical (Merck Ltd., Mumbai, India) in water, the solution was boiled for 10 min to remove any residual manganese(IV) ions, cooled, filtered using glass wool and diluted to 250 mL, and standardized using H.A Bright's procedure.[64] This solution was diluted stepwise to get 600 μg mL^{-1} for Indirect method and 0.1% for Direct method. Sulphuric acid (0.1M and 2M) were prepared by appropriately diluting required volumes of concentrated acid (S.D. Fine Chem., Mumbai, India, sp. gr. 1.84) with water to get the required concentrations. A 0.5 M solution of sodium hydroxide were prepared by dissolving about 10.0 g NaOH (Merck, Mumbai, India) in 500 mL of water and standardized against pure potassium hydrogenphthalate.[65] This was diluted to get 0.1M with water.

5.3. Standard drug solution

Pure sample of PGH was kindly supplied by Glenmark Pharmaceuticals, Mumbai, India, as gift. A 500 μg mL^{-1} stock standard solution was prepared by dissolving 50 mg of PGH in 0.1M H_2SO_4 and

made up to 100 mL with same acid in a calibrated flask. It was diluted to 50 μg mL^{-1} PGH for use in Indirect method. Forty mg of PGH were weighed accurately and dissolved in 0.1M NaOH in a 100 mL calibrated flask and diluted to the mark with the same solvent. The solution was then diluted with water to get a working concentration of 40 μg mL^{-1} for use in Direct method.

Two brands of PGH-containing tablets: Neoglit-30 (30 mg) (Novus Life Sciences Private Limited, Mumbai, India), Oglo-15 (15 mg) (Panacea Biotech., Mumbai, India) were procured from the local market.

5.4. General procedures

5.4.1. Procedure for bulk drug

5.4.1.1. Preparation of calibration graphs

5.4.1.1.1. Indirect Method

Different aliquots (0.25-5.0 mL, 50 μg mL^{-1}) of PGH solution were transferred into a series of 10 mL standard flasks by means of micro burette and the total volume was adjusted to 5.0 mL with 0.1M H_2SO_4. Two mL of 2M H_2SO_4 were added to each flask followed by 1 mL of 600 μg mL^{-1} $KMnO_4$ solution. The flasks were kept aside for 15 min with occasional shaking before diluting to the mark with water. The absorbance was recorded at 550 nm against a water blank.

5.4.1.1.2. Direct Method

Into a series of 10 mL volumetric flasks, 0.25-3.0 mL of 40 μg mL^{-1} PGH solution were buretted and the total volume was made up to 3.0 mL with 0.1M NaOH. To each flask was added 1 mL of 0.5M NaOH followed by 1 mL of 0.1% $KMnO_4$ solution. The flasks were kept aside for 10 min with occasional shaking and the volume was made upto the mark with water. The absorbance was recorded at 610 nm against the reagent blank.

Calibration graphs were prepared by plotting either decreasing absorbance values in Indirect method or increasing absorbance values in Direct method *versus* concentration of PGH. The unknown concentration was computed from the regression equation, derived using Beer's law data.

5.4.1.1.3. Procedure for tablets

A quantity of tablet powder containing 25 mg of PGH was accurately weighed into a 50 mL calibrated flask, added 30 mL of 0.1M H_2SO_4 and shaken for 20 min. Then, the volume was diluted to the mark with 0.1M H_2SO_4, mixed and filtered using a Whatman No. 42 filter paper. First 10 mL of the filtrate were discarded and a suitable aliquot of the subsequent portion (500 μg mL^{-1} in PGH) was diluted with 0.1M H_2SO_4 to obtain 50 μg mL^{-1} solution and the analysis was completed following the procedure described earlier by taking 3 mL aliquot in five replicates (Indirect method). In Direct method, a portion of the tablet powder equivalent to 20 mg of PGH was accurately weighed into a 100 mL beaker. The powder was extracted with three 10 mL portions of chloroform and each time the extract was filtered with a Whatman No. 42 filter paper. The filtrate was collected in 100 mL calibrated flask, chloroform was evaporated over a water bath and the residue was dissolved in 0.1M sodium hydroxide and diluted to the mark in a 100 mL calibrated flask with same solvent. The solution was diluted to 40 μg $mL^{-1}$and assayed by taking 2 mL aliquot (n = 5).

5.4.1.1.4. Procedure for placebo blank and synthetic mixture

A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was prepared by homogeneous mixing. A 20 mg portion was weighed and solution prepared as described under 'procedure for tablets' and then subjected to analysis.

A synthetic mixture was separately prepared by mixing 50 mg of pure PGH with 40 mg of placebo. Portions containing 25 mg or 20 mg of PGH were separately taken and solutions were prepared as described under "procedure for tablets" and analyzed following the general procedures, and the percentage recovery of PGH was computed.

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