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Research Article

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Development and Validation of new Colorimetric Method for the Estimation of Ketotifen and Pioglitazone

*Raval Kashyap¹, U.Srinivasa²

¹Research Scholar, Department of Pharmacy, Pacific University, Udaipur

²Head of Department, Srinivas College of pharmacy, Mangalore

ABSTRACT

Colorimetric methods have been developed for the estimation of Ketotifen & Pioglitazone. Here Methods were based on reaction involving the formation of green colored complex with Potassium permanganate. The maximum absorbance of the solution was measured at 616 nm & 602 nm against blank respectively. The calibration curve calculated obeys Beer's law over the concentration range of 25-45 & 20-40 µg/ml. The method was validated based on ICH guidelines. The high recovery and low relative standard deviation confirms the suitability of the method for determination Ketotifen & Pioglitazone. Hence it is useful for the routine analysis of Ketotifen and Pioglitazone.

Keywords: Ketotifen, Pioglitazone, 0.1N NaOH, UV spectrophotometric and validation parameters.

ARTICLE INFO

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*Corresponding Author

Raval Kashyap
Research Scholar, Department
of Pharmacy, Pacific
University, Udaipur
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1. Introduction

Ketotifen is used to minimize the frequency and severity of asthma attacks. Ketotifen relieves and prevents eye itchiness and/or irritation associated with most seasonal

allergies. It starts working within minutes after administering the drops. The drug has not been studied in children under 3. The mean elimination half-life is 12

hours. Its chemical name is 2-(1-methylpiperidin-4-ylidene)-6-thiatricyclo [8.4.0.0[^]{3, 7}] tetradeca-1(10), 3(7), 4, 11, 13-pentaen-8-one. Its Molecular formula & Molecular weight are C₁₉H₁₉NOS&309.42 respectively. Its structural formula is:

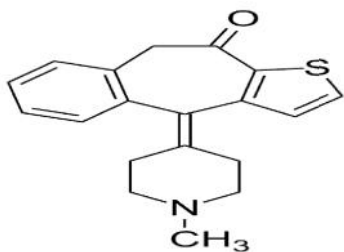


Figure 1

It is Soluble in water (10 mg/ml), DMSO and methanol. Ketotifen is a relatively selective, non-competitive histamine antagonist (H₁-receptor) and mast cell stabilizer. Ketotifen inhibits the release of mediators from mast cells involved in hypersensitivity reactions. Decreased chemotaxis and activation of eosinophils have also been demonstrated. Ketotifen also inhibits cAMP phosphodiesterase. Pioglitazone is an oral antihyperglycemic agent that acts primarily by decreasing insulin resistance. Chemically, pioglitazone is

2. Matirials and Methods

Preparation of standard stock solution of Ketotifen& Pioglitazone:

Stock solution of drugs were prepared by weighing accurately 100mg of pure drug into a 100ml volumetric flask and dissolved it and the volume was made up to mark with 0.1N NaOH to get a concentration of 1000 µg/ml. and further dilution were did to get 100 µg/ml.

Ketotifen:

Preliminary Investigation for Ketotifen:

To 2.5ml of the drug solution containing 30µg/ ml, 1 ml of 0.01% of Potassium permanganate was added & make up volume till 10ml with 0.1N NaOH then shake it and it will show green colour and measure the max. Corresponding reagent blank was prepared in the same manner omitting the drug. Shown in figure no 1.

STD Curve for Ketotifen:

From the working standard drug solution of 2.5, 3, 3.5, 4 & 4.5 ml (which gives 25-45µg/ml) drug solution was placed in 5 different 100 ml volumetric flasks. Into this 1.5 ml of 0.03% of Potassium permanganate was added then then volume was made up to 10ml with 0.1N NaOH then shake it & It will show green colour. Corresponding reagent blank was prepared in the same manner omitting the drug. The absorbance was measured against a reagent blank at 616 nm. Show in figure no. 2 and table no. 1.

Pioglitazone:

Preliminary Investigation:

To 2ml of the drug solution containing 20µg/ ml, 1 ml of 0.01% of Potassium permanganate was added & make up volume till 10ml with 0.1N NaOH then shake it and it will show green colour and measure the max. Corresponding

prepared as hydrochloride salt, which is identified as (±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2, 4-thiazolidinedionemonohydrochloride. Its Molecular formula & Molecular weight are C₁₉H₂₀N₂O₃S HCl& 392.90 respectively. [1] The structural formula is:

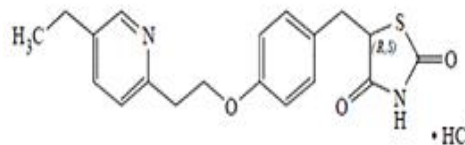


Figure 2

Pioglitazone hydrochloride is an odorless white crystalline powder that contains one asymmetric carbon in the thiazolidinedione moiety.

The synthetic compound is a racemate and the two enantiomers of pioglitazone interconvert in vivo. It is soluble in N, N dimethylformamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water, and insoluble in ether.²⁻³ some methods developed for same but here developed methods were new by KMNO₄.⁴⁻¹³ and all the methods were validated by ICH guideline. [14]

reagent blank was prepared in the same manner omitting the drug. Show in figure no. 3.

STD Curve for Pioglitazone

From the working standard drug solution of 2, 2.5, 3, 3.5 & 4 ml (which gives 20-40µg/ml) drug solution was placed in 5 different 10 ml volumetric flasks. Into this 2 ml of 0.03% of Potassium permanganate was added then volume was made up to 10ml with 0.1N NaOH then shake it & it will show green colour. Corresponding reagent blank was prepared in the same manner omitting the drug. The absorbance was measured against a reagent blank at 602 nm. Show in figure no. 4 and Table no. 2.

Validation for All Three Colorimetry:

Linearity:

A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance (by dilution of a standard stock solution) and using the proposed procedure. This method obeys the Beer- Lambert's law in the concentration range of 25-45 & 20-40µg/ml for Ketotifen& Pioglitazone respectively.

Accuracy:

Accuracy was established across the specified range of the analytical procedure. Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples.

Precision:

Repetability

Six times Replicates of 25 & 20µg/ml concentrations of Ketotifen& Pioglitazone respectively were prepared and

absorbance was measured at their respective max. SD and RSD were calculated.

Intraday Precision

Standard solutions containing 25, 30 and 35 µg/ml of Ketotifen/ 20, 25 & 30 for Pioglitazone were analyzed 3 times on the same day. The absorbance of solutions was measured at their respective max. SD and RSD were calculated.

Interday Precision: Standard solutions containing 25, 30 and 35 µg/ml of Ketotifen/ 20, 25 & 30 for Pioglitazone were analyzed 3 times on the different days. The absorbance

of solutions was measured at their respective max. SD and RSD were calculated.

Limit of Detection (LOD) & Limit of Quantitation (LOQ):

The LOD & LOQ are estimated from the set of 6 calibration curves used to determine method linearity.

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope}) \quad \text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

The entire validation summary shown in Table no. 3.

3. Result and Discussion

Ketotifen & Pioglitazone respectively was estimated based on the reaction of Potassium permanganate. The probable reaction takes place, resulting in the formation of greencolour which showed max at 616 nm & 602 nm

against blank respectively. The method obeyed Beer-Lambert's law in the concentration range of 15-45 µg/ml for Ketotifen & 20-40 µg/ml Pioglitazone respectively.

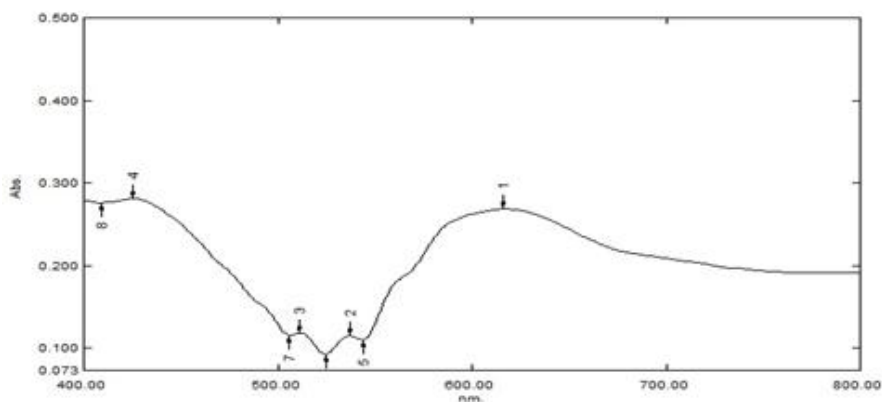


Figure 1: Potassium permanganate with Ketotifen

Table 1: STD CURVE For Ketotifen

SL. No.	Concentration of drug taken in µg/ml	Absorbance At 616 nm
1.	25	0.268
2.	30	0.474
3.	35	0.633
4.	40	0.790
5.	45	0.953

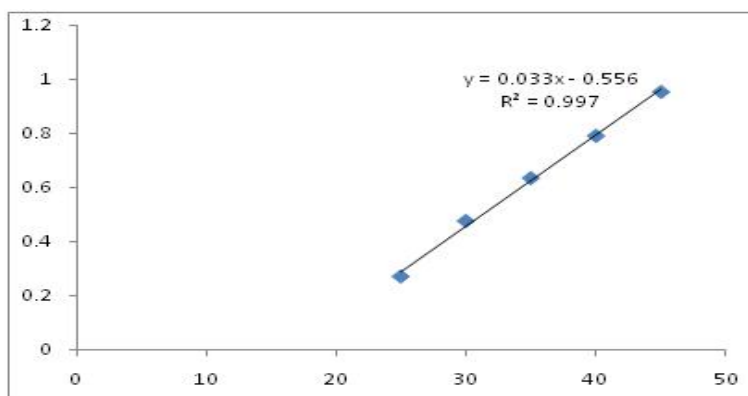


Figure 2: STD CURVE for Ketotifen

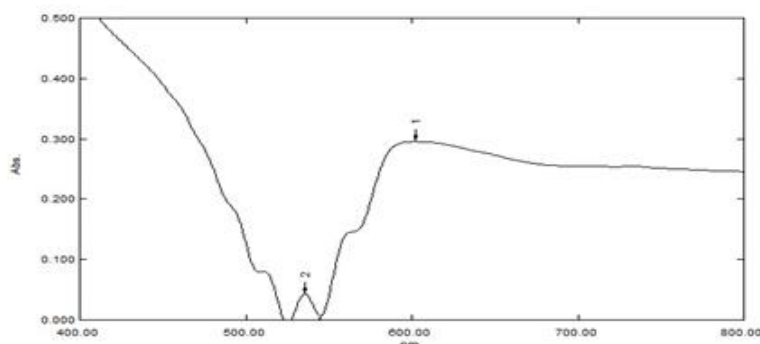


Figure 3: Potassium permanganate with Pioglitazone

Table 2: STD CURVE for Pioglitazone

SL. No.	Concentration of drug taken in $\mu\text{g/ml}$	Absorbance At 545 nm
1.	20	0.295
2.	25	0.456
3.	30	0.651
4.	35	0.832
5.	40	0.988

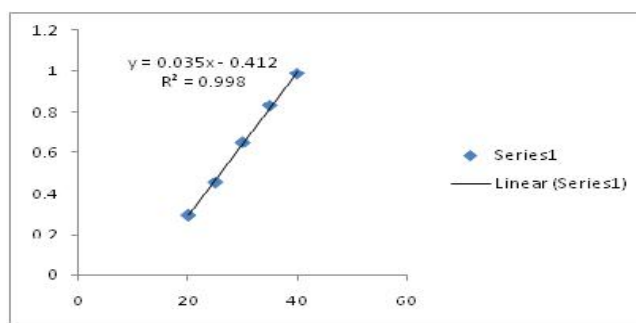


Figure 4: STD Curve for Pioglitazone

Table 3: The entire validation summary shown in Table no. 4.

Parameter	KETOTIFEN	PIOGLITAZONE
max (nm)	616	602
Beer's law limits ($\mu\text{g/ml}$)	25-45	20-40
Regression equation ($y=a+bc$)		
Slope (b)	0.0337	0.0352
Intercept (a)	-0.5566	- 0.4128
Correlation coefficient (r^2)	0.997	0.9987
precision		
Repeatability	0.265 \pm 0.005529	0.289 \pm 0.006735
Intraday	0.270 \pm 0.005508 0.480 \pm 0.002517 0.636 \pm 0.003512	0.293 \pm 0.006028 0.458 \pm 0.00755 0.652 \pm 0.00611
Interday	0.253 \pm 0.013577 0.465 \pm 0.012897 0.627 \pm 0.010536	0.281 \pm 0.013013 0.446 \pm 0.011533 0.642 \pm 0.010149
Limit of Detection	2.01	0.678
Limit of Quantitation	6.09	2.05

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