1, 2 Napthaquinone-4-sulphonic acid sodium salt Chromogen for Quantifying the Thiazolidinedione Class of drug in Human Plasma Spiked drug sample, bulk and marketed formulation as per Quality ICH guidelines.

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ABSTRACT

The current UV approach, which uses sodium salt 1, 2, napthaquinone-4-sulphonate, is comparatively easy to use, quick, and very sensitive for determining pioglitazone hydrochloride. The wavelength at which the pioglitazone hydrochloride exhibited absorption maxima was 455 nm. With a correlation value of 0.9996, the linearity range of pioglitazone hydrochloride was 5-120µg/ml. When pioglitazone hydrochloride was precisely measured, the result was determined to be less than 2. The outcomes of the suggested approach were deemed acceptable and suitable for estimating pioglitazone hydrochloride for regular quality control of the drug in the formulation and marketed formulation. A rapid and sensitive bio-analytical method was developed for the determination of Pioglitazone Hydrochloride in human plasma by protein precipitation extraction method. The ICH guidelines Q2R1 have been followed in the validation of this technique and M10 for bio-analytical method.

KEY WORDS: *Pioglitazone hydrochloride, 1, 2 NQS, method development as well as validation*

INTRODUCTION

Pioglitazone: [2, 3&7]

The pioglitazone IUPAC name 5-({4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl} methyl)-1, 3-thiazolidine-2, 4-Dione] is an anti-diabetic drug with chemical formula C19H20N2O3S. Pioglitazone belongs to the thiazolidinedione drug class and is an oral antidiabetic. Diabetes type 2 is treated with a medication known as pioglitazone. Completely soluble in methanol and ethanol; dissolves in dimethylformamide, somewhat in acetronitrile, and nearly insoluble in water and ether.

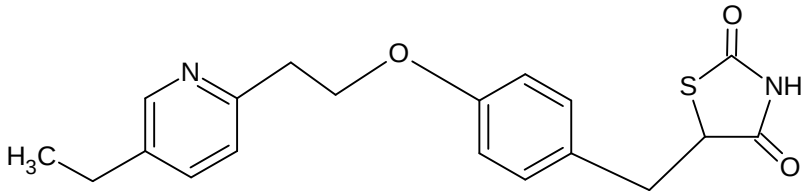

Figure1: Structure of Pioglitazone

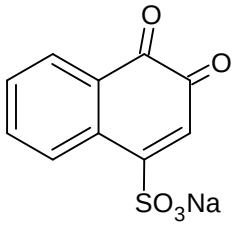

Figure2: Structure of the 1, 2 Napthaquinone-4-sulphonic acid sodium salt

MATERIALS ALONG WITH METHODS [5-10]

Chemicals wanted:

Pioglitazone, 1% 1, 2 Napthaquinone-4-sulphonic acid sodium salt (Folin's) , Methanol, Buffer solution of pH14 and Distilled Water

Required Instruments:

Double beam UV visible spectrophotometer, pH meter, sonicator, and weighing balance.

Reagent preparation:

PH 14 buffer solutions: After adding distilled water to the 100 ml volumetric flask, 4 grams of sodium hydroxide were taken and dissolved in it.

Preparation of 1% 1,2 Napthaquinone-4-sulphonic acid sodium salt reagent:

In a 100 ml volumetric flask, 0.1gm of the 1,2 napthaquinone-4-sulphonic acid sodium salt has been

added, then the remaining volume was filled with distilled water.

Making the Pioglitazone Standard Stock Solution:

A 10ml volumetric flask was filled with 0.01gm pure pioglitazone drug (a gift sample by Laurel Pharma Labs), which was then dissolved and made up with methanol. Pipette out 1.2ml from the standard stock solution.

The chemical reaction:

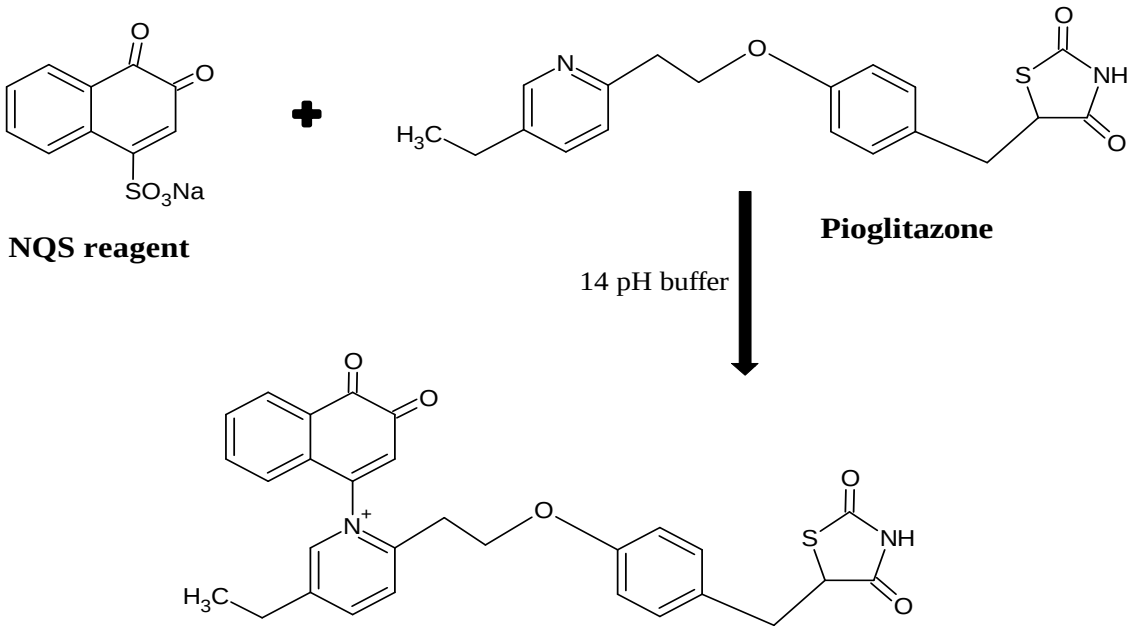

Figure3: Chemical reaction of NQS reagent with PG drug

Usually, when a chromogenic substance reacts with functional groups, a chemical transformation takes place, producing a coloured product. When 1, 2 Napthaquinone-4-sulphonate sodium (Folin's) reagents are treated with any amine-containing compound, the hydroxyl group is released when the sodium sulphonate group is replaced with an aromatic amine group (Figure3). This is how the reagents work.

Method optimization: *1, 14&25*

After taking 1.2 ml of the pioglitazone standard stock solution, 1ml of pH buffer mixture (14) was added. Add a 1ml of NQS Reagent next. As the reddish-orange colour finally appeared, it indicated the chromogenic response.

Following several tests, it was determined that the wavelength for the ideal trial was 455 nm, and the

absorbance was 1.3166(Figure4). Reddish orange color was discovered to be the optimal pH of pH14, after the color had settled for two hours.

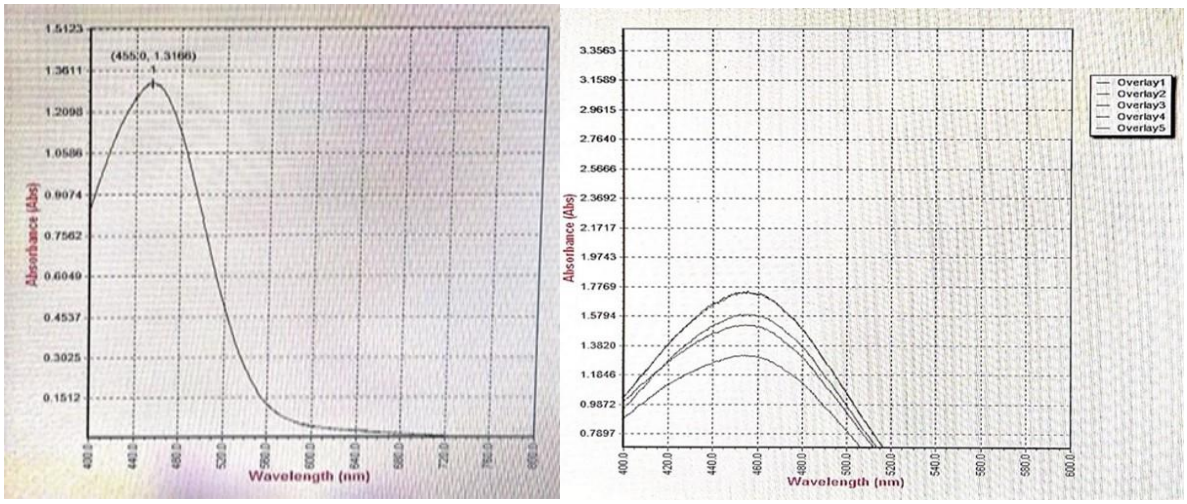

Figure4: Wavelength (nm) and overlay mode of linearity

Method optimization trials using pH values:

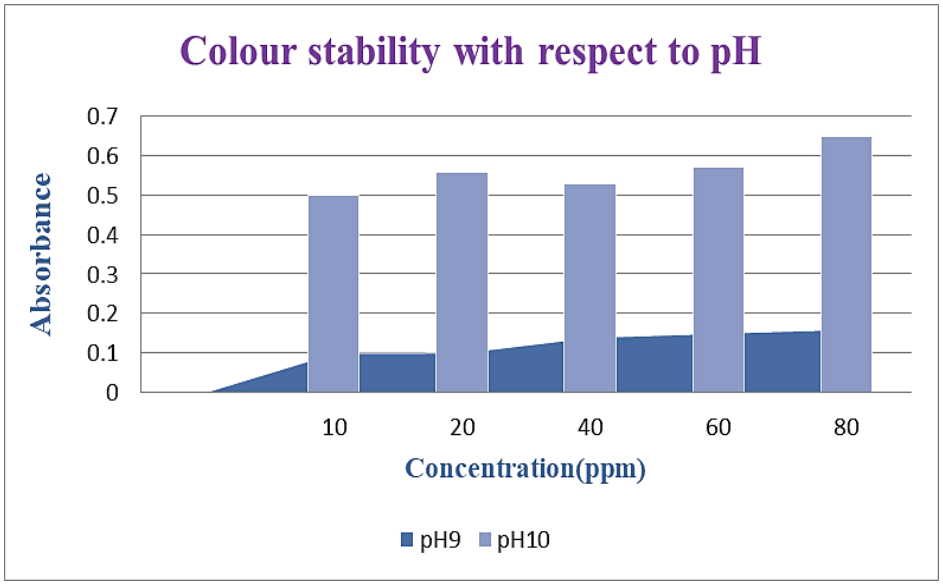

Figure5: Method optimization trials using pH values

The test was performed by using three different pH levels to optimize the colour. Finally, the method was optimized for pH14 value and the results of three different ranges as shown in the Figure5.

Colour Stability in Relation to Time:

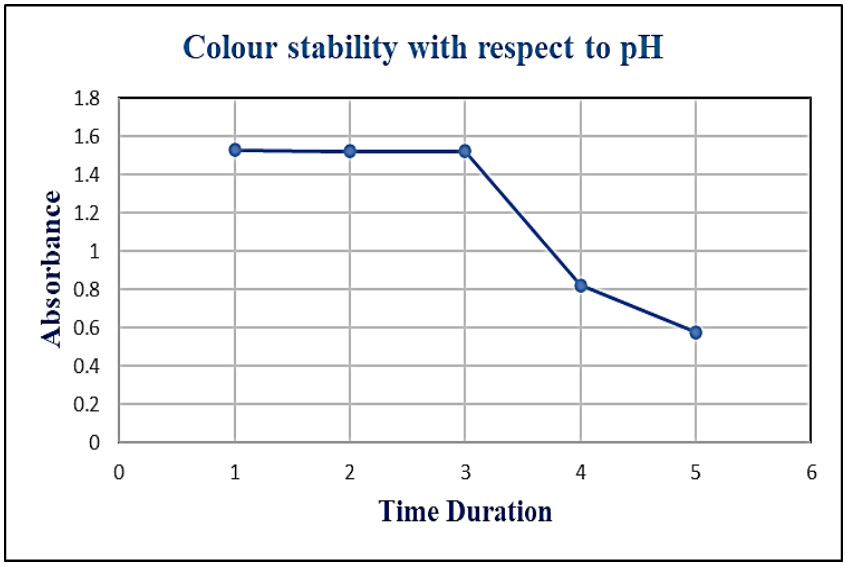

Figure6: Colour Stability in Relation to Time

The test was performed by checking the absorbance for every 30 minutes against a blank for the reagent, and it remained steady for two hours.

RESULTS AND DISCUSSION:

Method Validation Parameters: *12, 13, 15, 18-21*

The calibration curve was created by plotting concentrations on the X-axis against absorbance on the Y-axis, which assisted in estimating linearity. Based on the curve, the coefficient of correlation (r2) was determined to be 0.9996, which is still within the permissible range for the ICH.

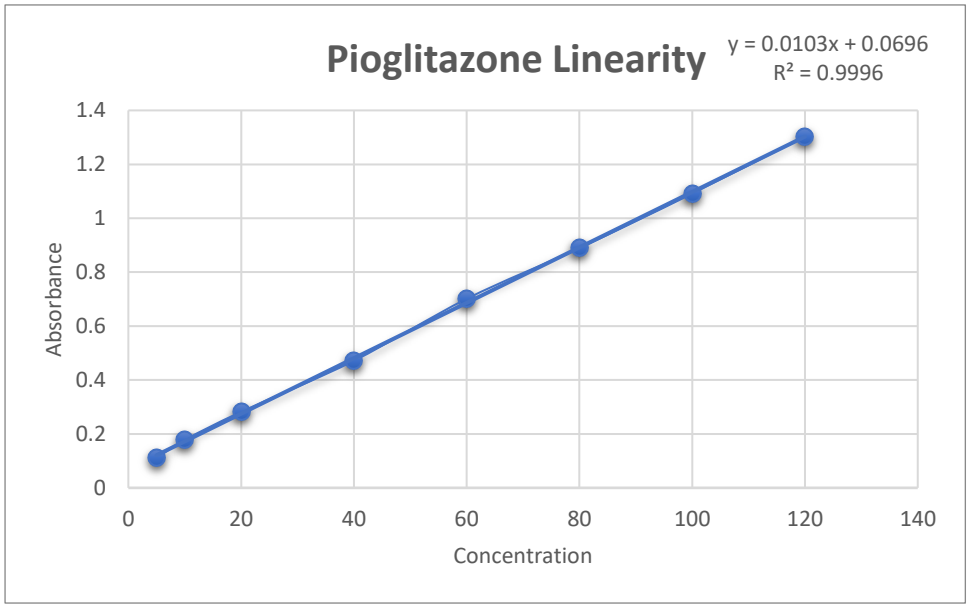

Figure7: Linearity data

The precision results of the preparation of six times 6 solutions are listed below. It was discovered that the pioglitazone's %RSD of repeatability was 0.04001 (Tab1). The relative standard deviation (RSD) of the intra-day precision % was found to be 0.02604 in the morning hours and 0.02903 in the evening (Tab2). Similarly, it was found that the inter-day precision deviation was 0.0406 on day 2 and 0.0142 on day 1 (Tab3). Based on the ICH standard, every single result fell within the permitted range.

Concentration (ppm)	**Absorbance**
80	0.889
80	0.8892
80	0.8893
80	0.8896
80	0.8898
80	0.8899
Mean	0.889466667
SD	0.000355903
%RSD	0.040013035

Table 1: Repeatability in precision

Concentration (ppm)	**Absorbance**	
80	0.8891	0.889
80	0.8892	0.8893
80	0.8894	0.8894
80	0.8895	0.8896
80	0.8896	0.8896
80	0.8897	0.8897
Mean	0.889417	0.889433
SD	0.000232	0.000258
%RSD	0.026046	0.02903

Table 2: Data for the intra-day precision

Concentration (ppm)	Absorbance	
	Day1	Day2
80	0.8896	0.8886
80	0.8897	0.8889
80	0.8896	0.8892
80	0.8898	0.8893
80	0.8899	0.8894
80	0.8896	0.8896
Mean	0.8897	0.889167
SD	0.000126	0.000361
%RSD	0.014217	0.040654

Table 3: Data for the inter-day precision

The Accuracy was performed by three degrees of additions of chemicals and reagents (50%, 100%, and 150%) in that 50% was done by spiking the 20ppm of standard to 40ppm of sample from that 1ml of the spiked standard solution and 1ml of the sample were pipette out into the 10ml volumetric flask. Similarly, 100% has been done by spiking 40ppm of standard to 40ppm of sample from that 1ml of the spiked standard solution and 1ml of the sample was pipetted out into a 10ml volumetric flask. Same as that 150% was done by spiking the 60ppm of standard to 40ppm of sample from that 1ml of the spiked standard solution and 1ml of the sample was pipetted out into a 10ml volumetric flask. For these three degrees of additions of chemicals add 1ml of pH 14 buffer, 1ml of NQS reagent and add water to make up the remaining volume, then the colour appears to be a complicated reddish-orange. The result of accuracy that is recovery range was determined to be 98.5–100.1%, which is within the ICH (98–102%) criteria.

Percentage Level	Sample Absorbance	Spiking Absorbance	Total Absorbance	% Recovery	Mean % Recovery
50% (40ppm +20ppm	0.4712	0.2821	0.7501	98.8%	98.5%
			0.7488	98.4%	
			0.7491	98.5%	

100% (40ppm +40ppm)	0.4712	0.4712	0.9390	99.2%	99.4%
			0.940	99.5%	
			0.942	99.9%	
150% (40ppm + 60ppm)	0.4712	0.7012	1.1726	100%	100.1%
			1.1734	100.1%	
			1.1742	100.2%	

Table 4: Accuracy data

The results showed that the Limit of Quantification (LOQ) and Limit of Detection (LOD) were 0.3455µg/ml and 0.11402µg/ml, respectively.

LOD	***LOQ***
0.114µg/ml	*0.3455µg/ml*

Table 5: LOD & LOQ data

Two separate analysts conducted the ruggedness analysis (Tab6); the result for the Analyst1 % RSD was found to be 0.026046 and Analyst2 got the %RSD 0.02903. We examined the robustness using two wavelengths (Tab7), +1 (%RSD 0.014217) along with -1(%RSD 0.04001), and the findings were within the acceptable ranges according to ICH guidance.

Concentration (ppm)	***Absorbance***	
	Analyst1	***Analyst2***
80	*0.8891*	*0.889*
80	*0.8892*	*0.8893*
80	*0.8894*	*0.8894*
80	*0.8895*	*0.8896*
80	*0.8896*	*0.8896*
80	*0.8897*	*0.8897*
Mean	*0.889417*	*0.889433*
SD	*0.000232*	*0.000258*
%RSD	*0.026046*	*0.02903*

Table 6: Ruggedness data

Concentration (ppm)	***Absorbance (nm)***	
	454nm	*456nm*
80ppm	*0.8879*	*0.8892*
80ppm	*0.8885*	*0.8895*
80ppm	*0.889*	*0.8899*
Mean	*0.888466667*	*0.889533*
SD	*0.000550757*	*0.000351*
%RSD	*0.061989614*	*0.03948*

Table 7: Robustness data

Pioglitazone dose form assay (Preparation of Pioglitazone Sample Solution):[27]

The marketed formulation assay: *Taken ten tablets (PIOZ15), each of which was weighed individually and then combined. Ten tablets were ground into a fine powder after being weighed. In a 10 ml volumetric flask, 10 mg of the corresponding pioglitazone tablets were weighed, dissolved with methanol using sonication, and then filled up with methanol. After preparing a further dilution to 80µg/ml, 1ml of the pH14 buffer solution and 1 ml of NQS reagent were added. By utilizing the formula "y = mx + c," the percentage of the assay was determined to be 102%.*

The percentage test was determined to be between 98 to 102%, which is within limits.

Bio-analytical Method Development: [12]

Analyzing analytes (drugs, metabolites, and biomarkers) of in biological samples is referred to as biological analysis. It is a multi-step process that includes sample collection, analysis, and data reporting. Protein precipitation extraction was the approach used to design the procedure. The steps taken to create this approach are listed below: 1ml of human plasma should be combined with 1ml of methanol for a simple extraction and 1ml of standard stock solution. The mixture should then be vortexed for two minutes and centrifuged for 15 minutes at 5000 rpm. After that, gather the liquid supernatant and add 1ml of pH14 buffer mixture and NQS reagent to that clear solution. Examine the mixture at 455 nm using a visible spectrophotometer. It was discovered that the resulting recovery rate was 99.8%.

Figure8 & Figure9: Colored complex & human plasma for Bio-analytical method development

CONCLUSION:

Pioglitazone HCL analytical method was developed and validated in bulk and marketed formulation by the simple, cost-effective, and precise method using the UV-Visible spectrophotometer as above-mentioned. Similarly using the UV-Visible spectrophotometer a bio-analytical method was developed. The developed method is helpful for routine analysis because the results are within the ranges.

***Acknowledgement*:** It needs to thank our beloved principle prof. M. Sumakanth mam and staff of the department of pharmaceutical analysis for giving this opportunity.

REFERENCES:

1. N.R. Doredla, “Method development and validation of forced degradation of pioglitazone hydrochloride by UV spectrophotometer” (international journal of pharm tech research), volume-4(4), pg.no:1750-1757, 2010.
2. A.P. Kulakarni, “Spectroscopic estimation of pioglitazone hydrochloride” (global journal of medical research), volume-12(20, pg.no:1-7, 2012.
3. Vijay Singh, “Method development of pioglitazone by UV spectrophotometer” (international journal of drug development and research) volume-6(4), pg.no-80-83, 2014.
4. Galen W Ewing,” Instrumental methods of chemical analysis”, Indian edition, 1970.
5. A. Dougla, F. Skoog, H. James, R.C. Stanley,” Introduction to UV visible spectroscopy, principles of instrumental analysis”, 6th edition, Pg.no: 114-122, 2007.
6. D. L. Pavia, M.L. Gary, A. James, S.K. George, “Ultraviolet spectroscopy, introduction to spectroscopy”, 4th edition, Pg.no:381-417, 2009.
7. The Indian Pharmacopeia 2018, volume 1, pp-1299-1300.

8. Atole, d.M. and H.H. RAJPUT, Ultraviolet Spectrophotometry and its pharmaceutical applications-a brief review. Asian J pharm clin. Res. 2018, 11(2), pp.59-66.

9. R.S. Shah, R.R. Shah, R.B. Pawar, And P.P. Gayakar, Uv-visible Spectrophotometry-a review. International journal of institutional pharmacy and life sciences, 2015. 5(5), pp.490-505.

10. A. Choudhary, The principle of ultra violet (UV) spectrophotometer. Medium. Com. 2017.

11. N. Erk and J. Biryol., "Voltammetric and HPLC techniques for the determination of paroxetine hydrochloride," Pharmazie, 2003, vol. 58, no. 10, pp. 699–704,.

12. M.M. Deshpande, V.S. Kasture, M. Mohan, And M.J. Chavan, Bioanalytical method development and validation: A review. In recent advances in analytical chemistry. Intech open. 2019.

13. H. Janzen, Forced degradation studies—comparison between ICH, EMA, FDA and WHO guidelines and anvisa's resolution RDC 53/2015. Master of drug regulatory affairs, rheinische friedrich-wilhelms-university of bonn, bonn. 2016.

14. M.K. Sharma, And M. Murugesan, Forced degradation study an essential approach to develop stability indicating method. Journal of chromatography and separation techniques, 2017, 8(1), pp.8-10.

15. C. B. Sekaran, V. H. Bind, M. R. Damayanthi, And A. Sireesha, Development and validation of UV spectrophotometric method for the determination of rivaroxaban. Der pharma chemica, 2013, 5(1), pp.1-5.

16. Y. R. Sharma "Introduction to UV visible spectroscopy, elementary organic spectroscopy", 1980, 4th edition, Pp.no: 280-339.

17. B.K. Sharma "Ultraviolet and visible spectroscopy, instrument method of chemical analysis", 23rd edition, Pg.no:68-192, 2004.

18. ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: text and methodology, 1996 Q2A.

19. R. A. Nash, A.H. Wachter, Pharmaceutical process validation, New York Marcel Dekker. 2003.

20. ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: text and methodology, 1995 Q2A.

21. K. Ishpreet, W. Sharad, PS. Harsharan, Development and Validation of UV Spectroscopic Method for Determination of Pioglitazone in Bulk and Pharmaceutical Dosage Form. Pharm Methods. 2015, 6(2):1-1.

22. PS. Harsharan, K. Ishpreet, S. Gunjan, Sodium Glucose Co-Transporters-2 (SGLT2) Inhibitors as a New Class of Anti-diabetic Drugs: Pharmacokinectics, Efficacy and Clinical Significance. Int J Pharm Sci Rev Res. 2015, 33(1): pp40-47.

23. Effat Souri, Hassan Jalalizadeh, Shahrooz Saremi, Development and Validation of a Simple and Rapid HPLC Method for Determination of Pioglitazone in Human Plasma and its Application to a Pharmacokinetic Study, Journal of Chromatographic Science, October 2008, Volume 46, Issue 9, Pages 809–812.

24. Ramalingam Peraman, Sasikala Mallikarjuna, Pravalika Ammineni, Vinod kumar Kondreddy., RP-HPLC Method Development and Validation for Simultaneous Estimation of Atorvastatin Calcium and Pioglitazone Hydrochloride in Pharmaceutical Dosage Form, Journal of Chromatographic Science, October 2014, Volume 52, Issue 9, Pages 1038–1042.

25. Pernica, Marek & Martiník, Jan & Bosko, Rastislav & Svoboda, Zdenek & Cwikova, Olga & Belakova, Sylvie & Benesova, Karolina. Ochratoxin A in roasted coffee marketed in Czech Republic: determination by liquid chromatography and fluorescence detection. Journal of Food and Nutrition Research. 2023, 62. 191-197.

26. M. M. Mabrouk, 'Three Spectrophotometric Methods for Determination of Alogliptin Benzoate and Pioglitazone HCl in Combined Tablet Dosage Form', *Analytical Chemistry Letters*, 2018, 8(3), pp. 368–378. doi: 10.1080/22297928.2018.1442246.

27. D. L. Dyer, Microbiological detection and identification system. 3,551,295A. *U.S. Patent.* 1970.

28. ICH, International Conference on Harmonization. Validation of Analytical Procedures: text and methodology Q2 (R1) *Harmonized Tripartite Guideline.* 2005.

29. AK. Moharana, M. Banerjee, S. Panda, JN. Muduli, Development and validation of UV spectrophotometric method for the determination of mesalamine in bulk and tablet formulation. *Asian J Pharm Clinical Res.* 2011; 4:71–73.

Table Captions:

Table 1: Repeatability in precision

Table 2: Data for the intra-day precision

Table 3: Data for the inter-day precision

Table 4: Accuracy data

Table 5: LOD & LOQ data

Table 6: Ruggedness data

Table 7: Robustness data