JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Application of Multi Component Analysis for the Estimation of Ranolzine and Metformin in Bulk and Combined Dosage Form by Using UV Spectrophotometer

Gangu Sreelatha,[1]* Chinnam Sucheta Krupalani [2], Dara Deepthi [3]

Department of Pharmaceutical Analysis, CMR College Of Pharmacy, Kandlakoya (V), Medchal Road, Hyderabad, India-50140

Article History:

Received 20 March 2024
Accepted 02 May 2024
Available online 15 June 2024

Citation:
Sreelatha G., Kruplani C. S., Deepthi D. Application of Multi Component Analysis for the Estimation of Ranolzine and Metformin in Bulk and Combined Dosage Form by Using UV Spectrophotometer **J Pharm Sci Bioscientific Res**. 2024. 12 (2): 68-72

For Correspondence:

Gangu Sreelatha.

Department of Pharmaceutical Analysis, CMR College Of Pharmacy, Kandlakoya (V), Medchal Road, Hyderabad, India.

(www.jpsbr.org)

ABSTRACT:

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method and simultaneous equation method for determination of Ranolazine and Metformin HCl. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an iso absorptive point and other being the λ-max of one of the two components. Simultaneous equation method uses the λ-max of both the drugs. Metformin HCl was determined at 235nm, Ranolazine was determined at 270nm and Iso absorptive wavelength was obtained at 250nm. Methanol and distilled water (50:50) was used as a solvent. The developed method was validated as per ICH guidelines. The Linearity of the calibration curve for each analyte in the desired concentration range was good (r2 > 0.989) by these methods. The method showed good reproducibility and recovery. The methods were successfully applied to pharmaceutical dosage form because of no interference.

KEYWORDS: Ranolazine, Metformin HCL, Methanol, Spectrophotometric, Absorbance Ratio Method, Iso absorptive point, Simultaneous equation method, ICH guidelines.

INTRODUCTION

Metformin HCl is used for co-morbid type 2 diabetes mellitus. Metformin HCl is chemically known as 3-(diamino methylidene)-1,1-dimethylguanidine; hydrochloride. It is the hydrochloride salt of the biguanide Metformin with antihyperglycemic and potential antineoplastic activities. Metformin inhibits complex I (NADPH:ubiquinone oxidoreductase) of the mitochondrial respiratory chain, thereby increasing the cellular AMP to ATP ratio and leading to activation of AMP-activated protein kinase (AMPK) and regulating AMPK-mediated transcription of target genes. This eventually prevents hepatic gluconeogenesis, enhances insulin sensitivity and fatty acid oxidation and ultimately leads to decrease in glucose levels.

Ranolazine is chemically known as N-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy) propyl] piperazin-1-yl] acetamide.

It is an orally available piperazine derivative with anti-anginal and potential antineoplastic activities. Ranolazine's mechanism of action for its anti-ischemic effects has yet to be fully elucidated but may involve the alteration of the trans-cellular late sodium current in the ischemic myocyte. By preventing the rise of intracellular sodium levels, Ranolazine may affect the transport activity of sodium-dependent calcium channels and prevent the calcium overload during myocardial ischemia, thereby preventing cellular injury. Combination of Ranolazine (RANO) and Metformin HCl is used for the treatment of patient suffering from chronic angina and co-morbid type 2 diabetes mellitus. [1-2]

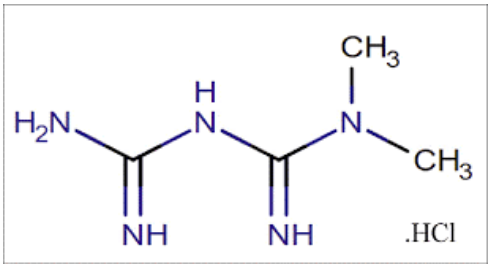

Figure 1: Metformin

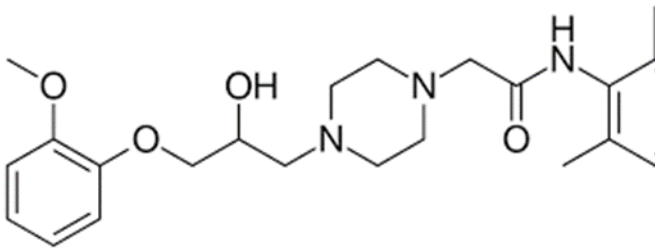

Figure 2: Ranolazine

In our literature review we found out that there are few methods available in both single form as well as in combination which are carried out via HPLC, UPLC, UV Spectrophotometry. There is no single method carried out by simultaneous equation method and Q absorbance ratio method for the given combination. Hence, the need of the hour was to obtain a method using these methods which is accurate, precise, robust, economical and time saving. (13-23)

Materials and Methods:

Apparatus and instrumentation:

A shimadzu 1800 UV-Vis double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Calibrated volumetric glassware (Borosil®) was used for the validation study.

Materials Reference standard of Metformin HCl and Ranolazine API were supplied as sample by CMR college of Pharmacy.

Method development:

Preparation of standard stock solution and test solution:

In both UV Spectrophotometric methods, 10mg each of Metformin and Ranolazine were weighed accurately and transferred into a 10 ml volumetric flask, dissolved in 5ml of Methanol and then volume was made up to mark with methanol to produce a stock solution containing 1000 µg/ml of Metformin and Ranolazine respectively. Aliquots of the stock solution were appropriately diluted with distilled water to obtain working standards of 100µg/mL solution. The linearity of the method was investigated by using concentration in range 2-10µg/ml and 10-50µg/ml for Metformin and Ranolazine respectively by diluting appropriate volume of the stock solution with distilled water.

Preparation of calibration curve:

The calibration curve was prepared by scanning test sample ranging from 2-10 µg/ml at 235 nm and 10-50µg/ml at 270 nm for Metformin and Ranolazine respectively. The calibration curve was constructed by plotting concentration of Metformin HCl and Ranolazine versus absorbance, and the regression equations were calculated.

Simultaneous Equation method:

The wavelength maxima of a Metformin HCl and Ranolazine were determined and found to be 235 nm (λ_1) and 270 nm (λ_2) respectively where there was no interference among the drugs.

By using the below equations, the concentrations in the samples were obtained

$$C_X = A_1a_{y2} - A_2a_{y1} / a_{x1}a_{y2} - a_{x2}a_{y1} \ldots\ldots 1$$

$$C_Y = A_1a_{x2} - A_2a_{x1} / a_{y1}a_{x2} - a_{y2}a_{x1} \ldots\ldots 2$$

Absorbance ratio method (Q-Analysis method):

Two wavelengths were selected for the method (270nm and 250nm) as one is the maximum wavelength for Ranolazine and the other is the isosbestic point respectively. Standard stock solutions were prepared separately. The stock solutions of both drugs were further diluted separately with distilled water to get series of standard solutions of 2-10 µg/ml for Metformin and 10-50 µg/ml for Ranolazine. The absorbances were measured at the selected wavelengths and absorptivity's (A 1%, 1cm) for both the drugs at both wavelengths were determined. (3-12)

The concentration of two drugs in the mixture can be calculated using following equations.

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/a_{x1} \ldots\ldots\ldots 1$$

$$C_Y = [(Q_M - Q_X) / (Q_Y - Q_X)] \times A_1/a_{y1} \ldots\ldots\ldots 2$$

Method validation:

UV method was validated in terms of linearity, precision and accuracy, robustness in accordance with ICH Q2 (R1) guidelines.

Linearity:

The linearity of an analytical procedure is its ability (within given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The calibration curve was constructed by plotting absorbance versus concentration and the linearity was evaluated by least square regression analysis. Calibration curve was prepared with appropriate volume of working standard solutions with the range of 2-10 µg/ml and 10-50 µg/ml respectively.

Precision:

Precision of the developed method was studied by performing intra-day and inter- day precision. The intraday precision was determined by performing six measurements of different concentration on the same day at different time interval. The inter-day precision of method was checked by repeating the study on two

consecutive days. The % RSD (Relative Standard Deviation) was calculated.

Accuracy:

Accuracy of the method was estimated by using standard addition method at three different levels by recovery experiments. The recovery is calculated from the test results as the percentage of analyte recovered by the assay. The known amounts of standard solutions of Ranolazine and Metformin were added to a pre evaluate test solutions of Ranolazine and mesalamine and the recovery was calculated.

Limit of detection:

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The limit of detection (LOD) of the drugs was derived by calculating the signal to noise ratio (S/N, i.e., 3.3. Limit of detection can be calculated using the following equation as per ICH guidelines. LOD = 3.3 x s/S Where, s = the standard deviation of response and S = Slope of calibration curve.

Limit of quantification:

It is the lowest concentration of an analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. The limit of quantification (LOQ) of the drugs was derived by calculating the signal to noise ratio (S/N, i.e., 10 for LOQ) using the following equation as per International Conference on Harmonization (ICH) guidelines. LOQ = 10 ×s/S Where, s = the standard deviation of response and S = Slope of the calibration curve.

Robustness:

The robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variation in method parameter and provide an indication of its reliability during normal usage.

Assay of combination dosage form:

Combination dosage form was prepared by equivalent to take 250 mg for both RANO and MET with common tablet excipients in appropriate amount. This mixture was diluted with methanol and distilled water to make concentration 40µg/mL and 6µg/mL for Ranolazine and Metformin respectively.

RESULTS AND DISCUSSION:

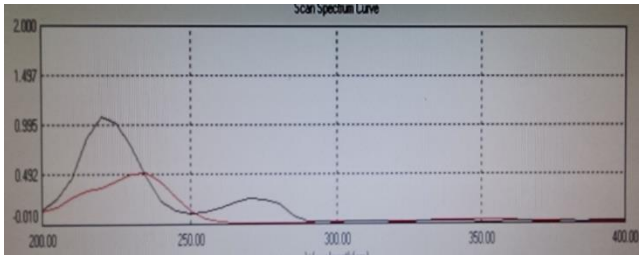

Figure 3: Iso-absorptive point of Ranolazine and Metformin

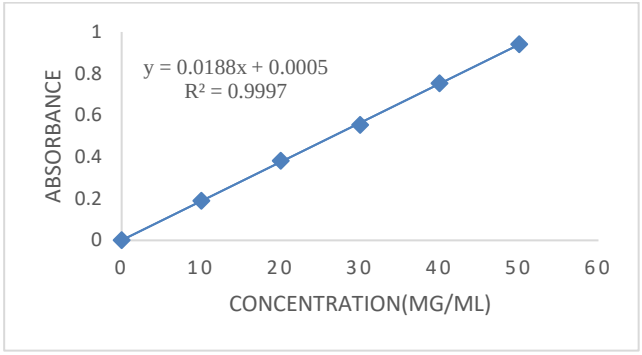

Figure 4: Ranolazine calibration graph

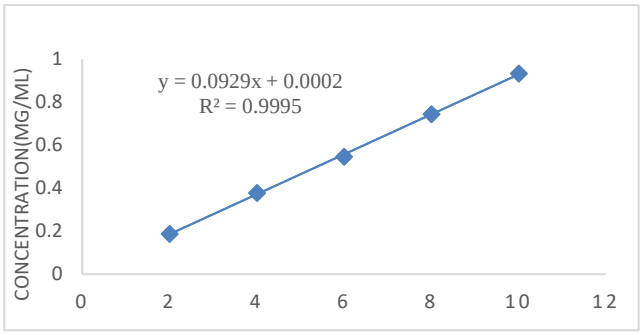

Figure 5: Metformin calibration graph

Table 1: Optical characteristics Data

Parameters	Method I Simultaneous equation method		Method II Q Absorbance ratio method	
	RANO	MET	RANO	MET
Working λmax	270nm	235nm	270nm	250nm
Beer's law limit	10-50µg/ml	2-10 µg/ml	10-50µg/ml	2-10 µg/ml
Correlation coefficient	0.9997	0.9995	0.9997	0.9995
Intercept	0.0003	0.0073	0.0002	0.0048

Slope	0.0096	0.081	0.0055	0.051
Regression equation	y = 0.0188x + 0.0005	y = 0.0929x + 0.0002	y = 0.0188x + 0.0005	y = 0.0929x + 0.0002
Intra-day(%RSD)	0.937	0.162	0.383	0.177
Inter-day(%RSD)	0.480	0.195	0.627	0.379
LOD(µg/ml)	1.33	0.205	1.04	0.075
LOQ	4.041	0.622	3.063	0.299

Accuracy:

Accuracy for the simultaneous method and Q-Analysis method were was estimated using the standard addition method at three different level by recovery experiment. For simultaneous method accuracy was 40.76537 %and 113.51513% for RANO and MET respectively. For Q-Analysis method accuracy was 40.76537 % and 113.51513% for RANO and MET respectively.

Table 2: Results for accuracy

Drug	Level of Recovery	Amount Added (µg/ml)	Amount estimated (µg/ml)	Recovery (%)	SD	%RSD
Ranolazine	50	1.5	4.489	99.32	0.00057	0.133
	100	3	6.01	100.47	0.00057	0.099
	150	4.5	7.58	101.7	0.001	0.137
Metformin	50	0.3	0.302	100.9	0.00057	0.078
	100	0.6	0.611	101.9	0.00057	0.058
	150	0.9	0.897	99.68	0.001	0.081

Precision:

Intraday and Interday precision was measured in terms of %RSD. The experiment was repeated 3 times a day for intraday and for 3 different days for inter-day precision.

Table 3: Precision values for the developed method

Method	Label claim	Amount found	%of drug content
I	RANO (20mg)	20.44	100.22
	MET (50mg)	49.99	99.98
II	RANO (20mg)	19.98	99.99
	MET (50mg)	50.02	100.04

Conclusion:

A new method was developed and validated for the determination of Ranolazine and Metformin using UV-Visible spectroscopy. The proposed method was found to be accurate, precise, simple, economic and rapid. The developed method can be applied for the assay of commercial tablets containing Ranolazine and Metformin in routine quality control analysis.

Acknowledgement:

We are thankful to CMR college of Pharmacy and the department of pharmaceutical analysis for providing the facilities such as lab, equipment and chemicals to carry out the work.

References:

1. PubChem. Ranolazine [Internet]. pubchem.ncbi.nlm.nih.gov. [cited 2024 Mar 3]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/56959
2. PubChem. Metformin [Internet]. pubchem.ncbi.nlm.nih.gov. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/4091
3. G.R. Chatwal, S.K. Anand, Instrumental method of Chemical Analysis, 5th ed, Himalaya Publishing House, New Delhi, 2011, 2.108-2.110p.
4. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle, Instrumental methods of Analysis,7th edn, CBS Publishers New Delhi 1986.159-161p.
5. Davidson AG (2002) Ultraviolet-visible absorption spectrophotometry. In BeckettAH, Stenlake JB, (4thedn), Practical Pharmaceutical chemistry. CBS Publishers and distributors, New Delhi, 275-278.

6. A.H. Beckett, J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th ed, Part 2, CBS Publisher and Distributor New Delhi, 2004, 275-278p.
7. Patil KM, Bodhankar SL (2005) High-performance thin-layer chromatographic
determination of lamotrigine in serum. J Chromatogr B Analyt Technol Biomed Life Sci 823: 152-157.
8. K.A. Connors, A textbook of pharmaceutical analysis, 3rd ed, John Wiley and Sons, 2007, 179-185p.D.G. Watson, A textbook for pharmacy students and pharmaceutical chemists, 2nd ed, Elsevier Churchill Livingstone, 2005, 89-91p.
9. D.A. Skoog, F.J. Holler, T.A. Nieman, Principal of Instrumental Analysis,5th ed, Thomson (Brook/Cole), 2004, 302-303p.
10. Dr. B.K. Sharma, Instrumental Method of Chemical Analysis, 28th ed, Goel Publishing house, 2012, S-72-S-74p.
11. GR. Chatwal, Sham K. Anand. Instrumental Methods of Chemical Analysis. 6th edition. Mumbai: Mrs. Meena Pandey;2011. Chapter 5, Visible Spectrophotometry and Colorimetry;2.108-2.109.
12. Ashutosh kar. Pharmaceutical drug analysis.3rd revised Edition. new age international publishers 2012.Pg no331-352.
13. Metformin drugs from Ip-Indian pharmacopoeia, volume 2, The Indian pharmacopoeia commission, Ghaziabad, Pg no 2544.
14. Jitesha Patel, Parth Patel, Rajashree Mashru. Novel UV-spectrophotometric & RP-HPLC method development and validation of simultaneous estimation of ranolazine and metformin HCL: A statistical analysis. Pharma Innovation 2020;9(7):50-57.
15. K Leela Bhaskar, D Sri Lakshmi, G Sumalatha, G Suji, K Anil Teja Kumar. Q-Analysis and Simultaneous Equation method for Estimation of Domperidone and Naproxen by UV Spectrophotometry in bulk and Tablet Dosage Form. Research J. Pharm. and Tech. 2020; 13(12):6050-6054. Doi: 10.5958/0974-360X.2020.01054.9
16. Zack J, Berg J, Juan A, Pannacciulli N, Allard M, Gottwald M, Zhang H, Shao Y, Ben-Yehuda O, Jochelson P. Pharmacokinetic drug–drug interaction study of ranolazine and metformin in subjects with type 2 diabetes mellitus. Clinical pharmacology in drug development. 2015 Mar;4(2):121-9.
17. Pettus J, McNabb B, Eckel RH, Skyler JS, Dhalla A, Guan S, Jochelson P, Belardinelli L, Henry RH. Effect of ranolazine on glycaemic control in patients with type 2 diabetes treated with either glimepiride or metformin. Diabetes, Obesity and Metabolism. 2016 May;18(5):463-74.
18. Maideen NM, Jumale A, Balasubramaniam R. Drug interactions of metformin involving drug transporter proteins. Advanced pharmaceutical bulletin. 2017 Dec;7(4):501.
19. Patel AB, Gol DA, Makwana RL, Vyas AJ, Patel AI, Patel NK, Faldu SD, Lumbhani AN. Analytical method for simultaneous estimation of ranolazine and metformin hydrochloride by validated RP-HPLC-DAD method. The Thai Journal of Pharmaceutical Sciences. 2022;46(3):287-92.
20. Wang L, Shang K, Feng T, Dong W, Wang F, Shen X. LC–MS/MS Method Assay for Simultaneous Determination of the Apixaban and Metformin in Rat Plasma: Assessment of Pharmacokinetic Drug–Drug Interaction Study. Journal of Chromatographic Science. 2023 Jul;61(6):522-9.
21. Darshan KN. Studies to Investigate the Influence of Ranolazine and Fluvastatin on the Pharmacokinetics and Pharmacodynamics of Glimepiride in Rats and Rabbits (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
22. Sowmya C, Kumar V, Adhikari B. Optimization and Analysis of Xanthan Gum and Hypromellose Combined Matrices for Extended Release of Ranolazine Using Quality by Design. International Journal of Pharmaceutical Investigation. 2022 Jan 1;12(1).
23. Development and validation of simultaneous estimation of canagliflozin and metformin by Q absorbance ratio method in its API and tablet formulation. Research Journal of Pharmacy and Technology [Internet]. 2022 [cited 2024 Mar 3];15(10).