VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 1284-1291 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Development of Stability Indicating RP-HPLC Method For Simultaneous Quantitation of Teneligliptin Hydrobromide Hydrate, Pioglitazone Hydrochloride and Metformin Hydrochloride in Bulk and its Tablet Dosage Form

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Received: 25th November, 2024; **Accepted:** 9th December, 2024; **Published online:** 13th December, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.129

Abstract: Following ICH guidelines, a forced degradation study using the RP-HPLC-PDA method has been designed and validated for simultaneous estimation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in bulk and recently approved triple FDC. Quantification was done by 0.025 M KH_2PO_4 Buffer: Methanol: Acetonitrile (50:25:25 % v/v/v) pH 3 at 225 nm wavelength and 1ml/min flow rate. Linearity obtained was 2-6 µg/ml for Teneligliptin hydrobromide hydrate, 1.5- 4.5 µg/ml for Pioglitazone hydrochloride and 50-150 µg/ml for Metformin hydrochloride. % Relative standard deviation was less than 2% for precision. Analysis of degradation behaviour under different stress conditions like acidic, basic, oxidative, photolytic and thermal, demonstrates the specificity of the proposed method.

Keywords: RP-HPLC, Degradation study, Teneligliptin hydrobromide hydrate, Metformin hydrochloride, Pioglitazone hydrochloride

Citation: Kagarana Chhayaben S., Patel Kunal N. and Patel Advaita B.: Development of stability indicating RP-HPLC method for simultaneous quantitation of teneligliptin hydrobromide hydrate, pioglitazone hydrochloride and metformin hydrochloride in bulk and its tablet dosage form. Intern. J. Zool. Invest. 10(2): 1284-1291, 2024.

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Introduction

Diabetes is not a single disease rather it is a heterogenous group of syndromes marked by a rise in blood glucose levels by relative or absolute deficiency of insulin. The American Diabetes Association (ADA) recognizes 4 clinical classifications of diabetes: Type 1 diabetes, type 2 diabetes, gestational diabetes and diabetes due to other causes like genetic defects or medications (Bakshi and Singh, 2002; Michelle *et al*., 2012; Hotha *et al*., 2013).

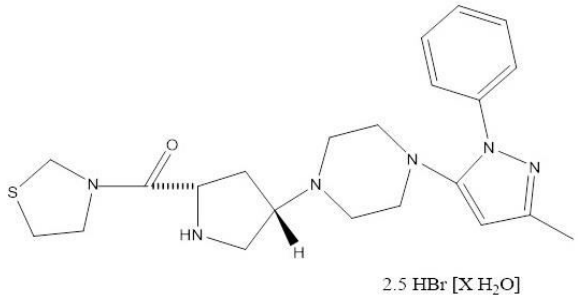

Fig. 1: Structure of Teneligliptin hydrobromide hydrate.

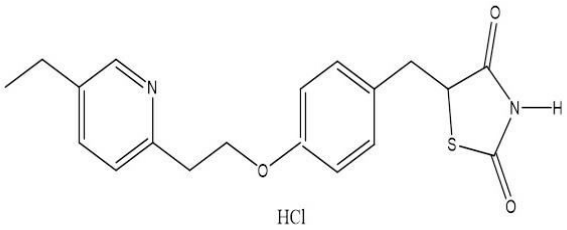

Fig. 2: Pioglitazone hydrochloride structure.

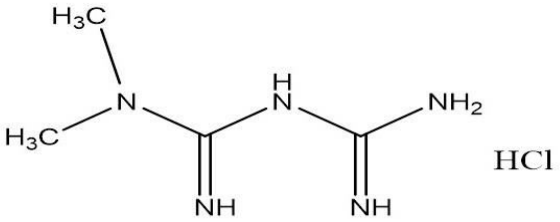

Fig. 3: Metformin hydrochloride structure.

Teneligliptin hydrobromide hydrate (TEN) is chemically [(2*S*,4*S*)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thia-zolidin-3-yl) methanone; hydrate; pentahydrobromide. The chemical formula is $C_{22}H_{30}N_6OS$, 2.5HBr, XH_2O. Pioglitazone hydrochloride (PIO) has the chemical formula $C_{19}H_{21}ClN_2O_3S$ and its chemical name is 5-[[4-[2-(5-ethyl pyridine-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione; hydrochloride. Metformin hydrochloride (MET) is chemically 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride. Its chemical formula is $C_4H_{12}ClN_5$. Figures 1, 2 and 3 show the chemical structures of TEN, PIO and MET, respectively. In Indian pharmacopoeia, all three drugs are official (European Pharmacopoeia, 2004; Indian Pharmacopoeia, 2018).

The FDA recently approved a combination of TEN, PIO and MET. The literature review leads to the conclusion that different methods like HPLC, HPTLC, UV, LC/MS/MS were reported for

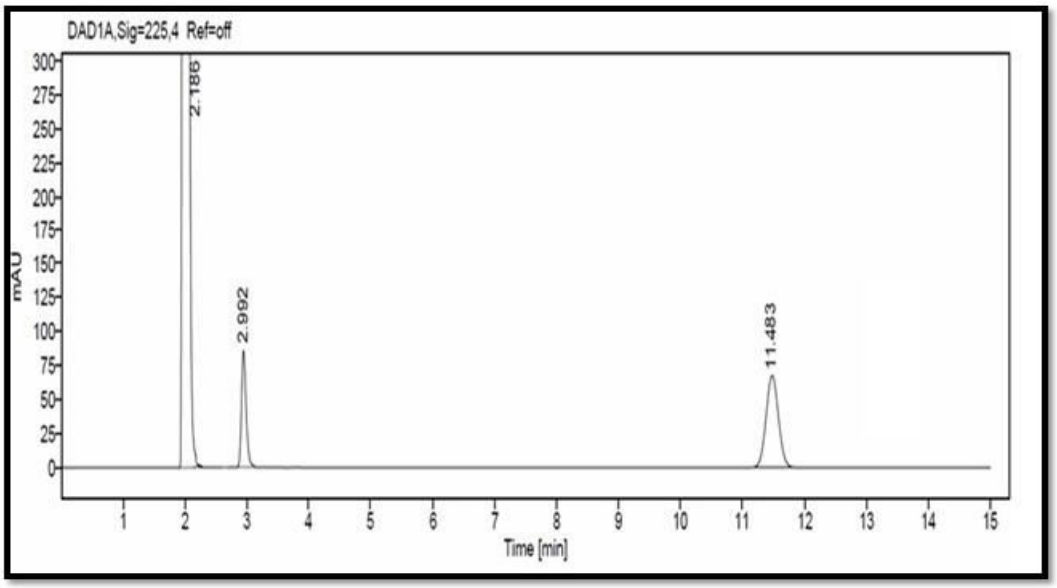

Fig. 4: HPLC Chromatogram of Metformin hydrochloride- 1000 µg/ml, Teneligliptin- 40 µg/ml and Pioglitazone hydrochloride- 30 µg/ml in Acetonitrile: methanol: 0.025 M KH_2PO_4 Buffer (25:25:50 %v/v/v) pH 3 adjusted by OPA (Optimized condition).

determining TEN, PIO and MET in either a single drug or in combination with other medications. There are no existing ways to quantitatively determine them simultaneously in its combination (Kapil and Sharma, 2019; Nataraj *et al*., 2019; Balaji *et al*., 2020; Israa *et al*., 2020; Mahmoud *et al*., 2020; Pramila *et al*., 2020; Suddhasattya *et al*., 2020; Tammisetty *et al*., 2021; Maneesha *et al*., 2021; Nazar Mustaf *et al*., 2021; Syed *et al*., 2021; Cristina *et al*., 2021; Nasr *et al*., 2023).

Following ICH guidelines, a forced degradation study using the RP-HPLC-PDA method has been designed and validated for simultaneous estimation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in bulk and recently approved triple FDC.

Materials and Methods

Materials, reagents and pharmaceutical formulation

Gift samples of TEN, PIO and MET standards were acquired from reputed pharmaceutical companies in Gujarat, India. HPLC-grade methanol, acetonitrile and water were purchased from E. Merck Chem. Limited, Mumbai, India. All other required chemicals were of analytical grade (CYNOR Pharma Private Limited).

Instrumentation

Agilent 1200 infinity II LC chromatographic system was used as a High-Performance liquid chromatograph equipped with a 1260 Quat Pump VL and PDA detector. Sapphires Column C_{18} (250 mm × 4.6 mm, 5 µm particle size) was used. EZ Chrome software was used for data acquisition and integration. (Marie *et al*., 2023).

Mobile phase selection and optimization

After several mobile phase trials, a combination of 0.025 M KH_2PO_4 Buffer, Methanol and Acetonitrile in the ratio of 50:25:25 % v/v/v pH 3 adjusted by 1% Orthophosphoric acid at 1 ml/min flow rate offered improved separation and quantification with nice peak shape, high number of theoretical plates and asymmetry for simultaneous estimation of TEN, PIO and MET in its triple FDC. Figure 4 displays the HPLC chromatogram of TEN, PIO and MET at this optimized condition (Morosi *et al*.,

2022).

Selection of detection wavelength

At 225 nm, the combination of three drugs produced a satisfactory response.

Procedure

Preparation of mobile phase

0.025 M KH_2PO_4 Buffer was prepared by dissolving 1.7 g KH_2PO_4 in HPLC grade water in 500 ml. pH of the solution was adjusted to 3.0 by 1% orthophosphoric acid. The final mobile phase was prepared by taking 500 ml of prepared buffer, 250 ml of Acetonitrile and 250 ml of Methanol and mixed for 5 min under sonication. This solution was filtered from 0.45 µ Whatman filter paper and was loaded into the system (Duddukuru *et al*., 2020).

Preparation of solutions

20 mg TEN, 15 mg PIO and 500 mg MET standards were accurately weighed and transferred in different 100 ml volumetric flasks to prepare 200 µg/ml TEN, 150 µg/ml PIO and 5000 µg/ml MET standard stock solutions, respectively. 2ml from all three standard stock solutions was taken respectively and transferred to the same 10 ml volumetric flask and volume was made up to the mark with methanol to prepare 40 µg/ml TEN, 30 µg/ml PIO and 1000 µg/ml MET stock solution, respectively (Mane *et al*., 2022).

Preparation of sample solution

Weighed 20 tablets accurately and then the average weight was determined. After that, the finely crushed tablet powder equivalent to 2 mg TEN, 1.5 mg PIO and 50 mg MET was weighed and transferred to a 100 ml volumetric flask. The solution was sonicated for 15 min and made up the volume with methanol. The solution was filtered through Whatman filter paper no. 41. 2 ml from this sample stock solution was taken and transferred to a 10 ml volumetric flask and made up the volume to the mark with the methanol to get 4 µg/ml TEN, 3 µg/ml PIO and 100 µg/ml MET concentration. 20 µl of this solution was injected for assay analysis.

Study of Stress testing

The sample solutions were subjected to different degradation conditions like acid (0.1 N HCl at 70℃ for 1 h), base (0.1N NaOH at 70℃ for 1 h), oxidation (3% H_2O_2 at 70℃ for 1 h), photolytic (36 h at 254 nm) and thermal (3 h at 70℃). Once the degradation process was finished, the chromatograms under optimized conditions were observed and % degradation was calculated.

ICH method validation

As per ICH guidelines, different validation parameters like Precision, Accuracy, Linearity, Range, Limit of detection, System suitability, Limit of Quantitation, Robustness, Specificity and System suitability were checked using 4 µg/ml TEN, 3 µg/ml PIO and 100 µg/ml MET concentration.

Results and Discussion

Stress testing

TEN, PIO and MET experience varying levels of degradation under different conditions in tablet formulation. Obtained chromatograms are given in Figures 5-9. The results of forced degradation studies are shown in Table 1.

Validation method

The developed method was validated for different parameters as per ICH guidelines. Obtained results of validation parameters are summarized in Table 2.

Analysis of a marketed formulation: Table 3 provides % assay of the sample solution.

Method conclusion: Zita-Pio-Met 500 tablet is recently approved triple FDC. So, no methods are available for simultaneous quantification of them. The current research presents the first approach dealing with the development of stability-indicating RP-HPLC assay. A specific, simple, sensitive and cost-effective forced degradation method was developed which is suitable for

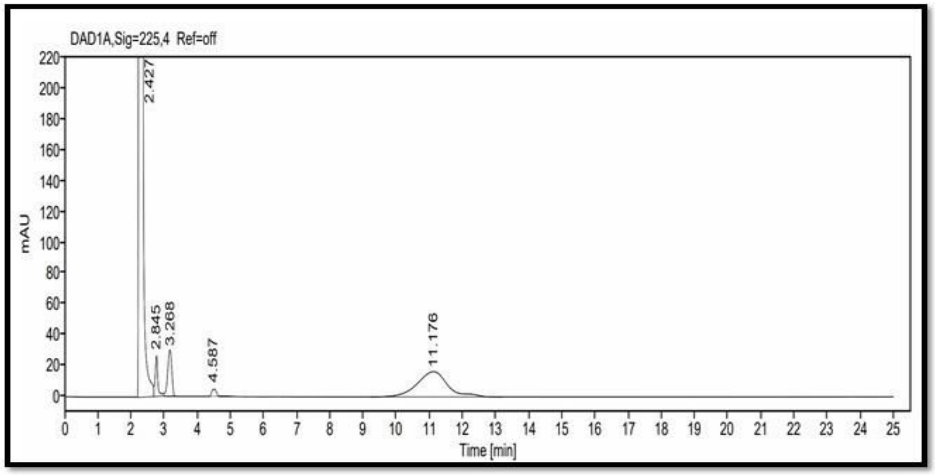

Fig. 5: Acid degradation chromatogram in 0.1 N HCl, 70°C,1 h.

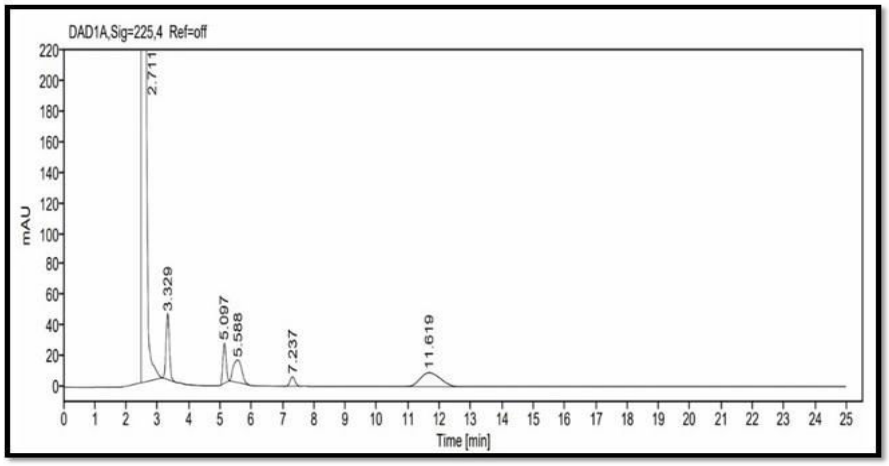

Fig. 6: Base degradation chromatogram 0.1 N NaOH,70°C, 1 h.

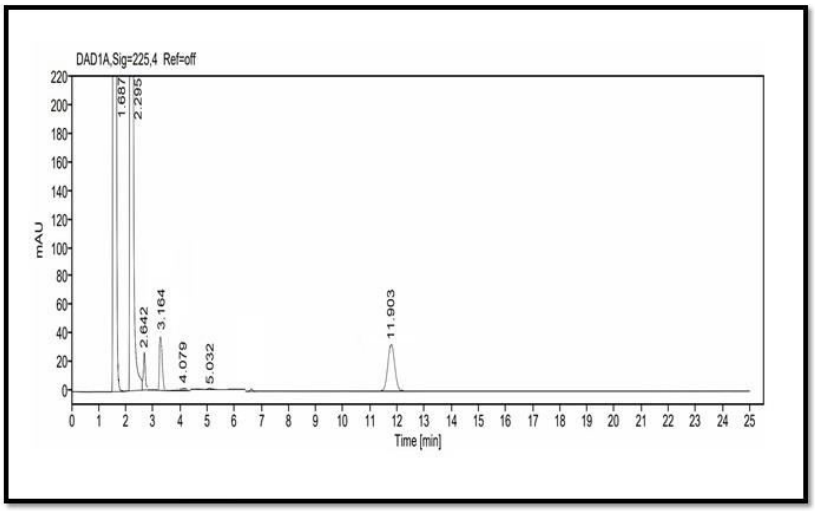

Fig. 7: Oxidative degradation chromatogram in 3% H_2O_2, 70°C.

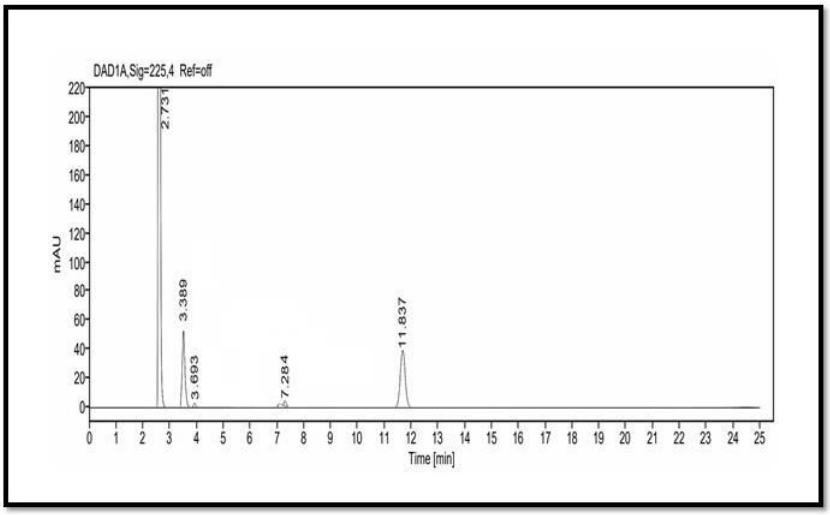

Fig. 8: Photodegradation chromatogram in 36 h.

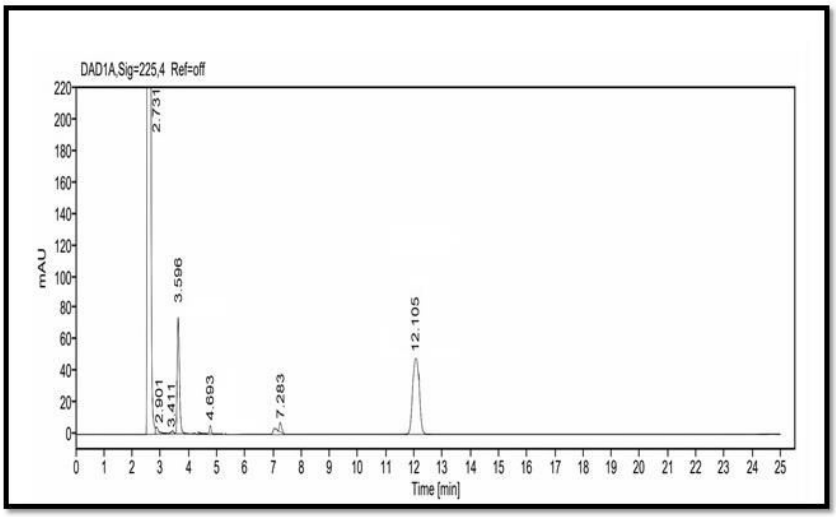

Fig. 9: Thermal degradation chromatogram in 3 h, 70℃.

Table 1: Outcomes of stress testing study

Degradation	Condition	% Degradation		
		TEN	PIO	MET
Acid	0.1 N HCl at 70℃,1 h	13.59	11.66	11.16
Base	0.1 N NaOH at 70℃, 1 h	19.93	18.64	12.57
Oxidation	3% H_2O_2, 1 h	17.67	18.83	15.07
Photo	3 hrs at 70℃	8.90	10.85	13.98
Thermal	36 hrs at 254 nm	14.59	11.98	10.20

Table 2: Validation Parameters summary

S. No.	Parameters	TEN	PIO	MET
1	**Linearity Range**	2.0-6.0 µg/ml	1.5 – 4.5 µg/ml	50-150 µg/ml
2	**Regression Equation (y = mx + c)**	y = 935 x+ 226.6	y = 3351.5 x - 327.4	y = 4437.3 x + 5240.6
3	**Correlation coefficient (R^2)**	0.9993	0.9983	0.9979
4	**Accuracy (% Recovery)**	99.35 % -100.06 %	99.18 % -100.31 %	99.35 % -100.12%
5	**LOD (µg/ml)**	0.473	0.417	4.718
6	**LOQ(µg/ml)**	1.432	1.189	14.298

Table 3: % Assay of marketed sample

Tablet	Zita Pio Met 500		
Label claim	**TEN (20 mg)**	**PIO (15 mg)**	**MET (500 mg)**
Assay (% Mean ± S. D.)	99.23 ± 0.14	98.51 ± 0.19	99.15 ± 0.49

simultaneous determination in the presence of degradants.

The purpose of the present research was to develop cost-effective, less time-consuming method which was also complied with ICH guidelines. So, it can be applied for regular analysis of the simultaneous determination of TEN, PIO and MET.

References

Bakshi M and Singh S. (2002) Development of validated stability-indicating assay methods--critical review. J Pharm Biomed Anal. 28(6):1011-1040.

Balaji N and Sayeeda S. (2020) Assessment of Related Substances in Pioglitazone hydrochloride by HPLC method. Challenges Adv Pharmaceut Res.1: 65-79.

Duddukuru P, Syed A and Pigili R. (2020) Simultaneous determination of alogliptin and pioglitazone in human plasma by a novel LC-MS/MS method. Curr Pharm Anal. 16: 564-577.

European Pharmacopoeia (2004) Council of Europe, Strasbourg 5th edn., volume II, pp 1660-1662.

Hotha K, Reddi SK Raju VK and Ravindranath LK. (2013) Forced degradation studies: a practical approach- overview of regulatory guidance and literature for the drug substances and drug products. Int Res J Pharm. 4:78-83.

Indian pharmacopoeia (2018) Government of India, Ministry of Health and Family Welfare, Ghaziabad, pp. 2175-2177.

Israa H, Al-shdefa R, Mohammad H and Abu Dayyi W. (2020) Stability indicating RP-HPLC method development for simultaneous estimation of Alogliptin, Pioglitazone, and Metformin in pharmaceutical formulations. Acta Pol Pharm Drug Res. 77: 549-562.

Kapil R and Sharma P. (2019) Development and Validation of a HPLC method for the determination of Metformin hydrochloride, Nateglinide and Pioglitazone hydrochloride in multicomponent formulation. Asian J Res Chem. 14:15-20.

Mahmoud S, Sobhy El-Adl M, Mohamed B and Amira H. (2020) Rapid RP-HPLC method for simultaneous estimation of Metformin, Pioglitazone, and Glimepiride in human plasma. Acta Chromatogr. 32: 16-21.

Mane S and Mahaske M. (2022) Development and stability indicating RP-HPLC method for simultaneous estimation of Metformin HCl and Teneligliptin HBr hydrate in bulk and pharmaceutical dosage form. Indo American J Pharmaceut Sci. 9(6): 499-518.

Maneesha K, Mounika R, Sultana S, Nasimul H, Sujon A, Prashanthi S, Islam S, Nasim Aktar S and Shanawaz K. (2021) Method development and validation of Alogliptin and Pioglitazone by RP- HPLC method in bulk and its marketed dosage form. World J Pharm Res. 10: 782-798.

Marie A, Hammad S, Salim M, Elkhodary M and Kamal A. (2023) Deduction of the operable design space of RP-HPLC technique for the simultaneous estimation of Metformin, Pioglitazone and Glimepiride. Sci Rep. 13: 1-13.

Michelle AC and Richard Finkel JA. (2012) Pharmacology. Lippincott's illustrated reviews, pp. 301.

Miralles-Cardiel E, Silva-Abreu M, Cristina A and Isidre C. (2021) Development and validation of an HPLC–MS/MS method for Pioglitazone from nanocarriers quantitation in ex vivo and in vivo ocular tissues. Pharmaceutics 13: 1-15.

Morosi L, Matteo C, Meroni M, Ceruti T, Nerini L, Bello E and Frapolli R. (2022) Quantitative measurement of Pioglitazone in neoplastic and normal tissues by AP-MALDI mass spectrometry imaging. Talanta 237: 122918.

Nasr M El-Abasawy, Ahmed EL-Olemy, Mohamed M. Sharaf El-din and Ali F. (2023) Determination of Alogliptin benzoate and Pioglitazone hydrochloride in their dosage forms, validation and stability-indicating studies using RP-HPLC method. Octahedron Drug Res. 2: 1-10.

Nataraj KS, Srinivasa Rao AS, Nagamani KV, Divya P and Renukachandrasekhar B. (2019) Analytical method development and validation of Metformin, Pioglitazone and Glimepiride by RP-HPLC in tablet dosage forms. J Pharm Innov. 8: 266-273.

Nazar Mustaf M and Anurekha J. (2021) Simultaneous estimation of Metformin hydrochloride, Pioglitazone hydrochloride and gliclazide by validated RP HPLC method in solid dosage form. Int J Pharm Pharm Sci. 4: 72-76.

Pramila T, Agrwal A and Sharma M. (2020) Development and validation of Pioglitazone hydrochloride in bulk and pharmaceutical formulations by RP-HPLC method. World J Pharm Res. 9:1966-1977.

Suddhasattya D, Souvik B, Anjan D, Shahreja P, Tabassum H, Achintya S, Manik G and Tanushree K. (2020) Simultaneous pharmacokinetics estimation of Nateglinide and Pioglitazone by RP-HPLC: Computational study to unlock the synergism. J Chromatogr Sci. 58: 309-322.

Syed R and Kantipudi R. (2021) New validated method for the estimation of Pioglitazone and Rosiglitazone using RP HPLC. J Pharm Res Int. 33: 254-262.

Tammisetty M, Challa B and Puttagunta S. (2021) A novel analytical method for the simultaneous estimation of Remogliflozin and Metformin hydrochloride by UPLC/PDA in bulk and formulation application to the estimation of product traces. Turk J Pharm Sci. 3: 1-21.