DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF TENELIGLIPTIN HYDROBROMIDE AND METFORMIN HYDROCHLORIDE IN ITS BULK AND DOSAGE FORM

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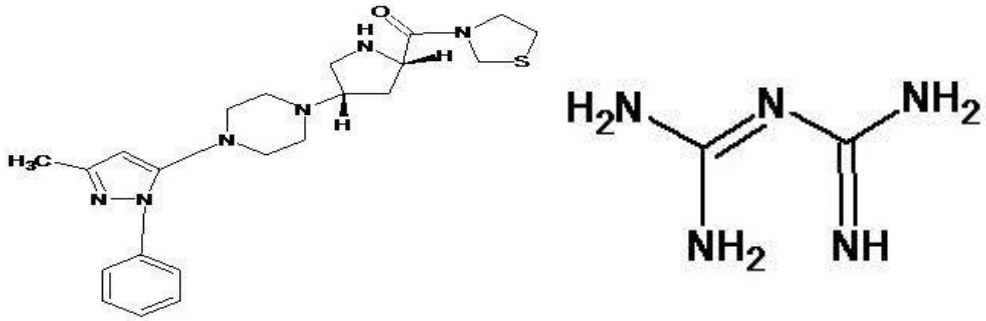
Abstract: Advantages such as simple sample preparation steps with no time-consuming extraction and separation lead to the more frequent use of simultaneous estimation procedure in case of the multicomponent pharmaceutical dosage forms which are accurate and precise. In the present study, a UV Spectrophotometric method has been developed and validated for the simultaneous estimation of teneligliptin hydrobromide and metformin hydrochloride in bulk and tablet dosage form. The estimation was performed by using two methods namely; Simultaneous equation method and Absorbance ratio method. Both the spectrophotometric methods employed preparation of dilutions using distilled water. In simultaneous equation method, absorbance was measured at 230nm and 245nm for both the drugs whereas, in absorbance ratio method the absorbance was measured at 230nm and 240nm for both the drugs. The calibration plot was found to be linear between concentration range 2-12 µg/ml for metformin and 5 -55µg/ml for teneligliptin with R^2 = 0.999 for both the drugs. The amount of drugs estimated by both the methods was in good agreement with the label claim. The method was validated according to the ICH guidelines. Therefore, both the developed methods can be used for routine quality control analysis of Teneligliptin and metformin in bulk and pharmaceutical formulation.

***Keywords*: Teneligliptin HBr, Metformin HCl, Simultaneous equation, Absorbance ratio.**

I. INTRODUCTION

Teneligliptin hydrobromide hydrate (TEN) is {(2S,4S)- 4-[4-(3-Methyl-1-phenyl-1*H*-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3-thiazolidin-3-yl) methanone hemipentahydrobromide hydrate (Sharma, Surendra, 2016), a oral dipeptidyl peptidase inhibitor (DPP-4). DPP-4 inactivates incretin hormones (glucagon-like peptide-1; GLP-1 and glucose-dependent insulinotropic polypeptide; GIP) which are responsible for enhancing insulin secretion. It is indicated for the management of type 2 diabetes mellitus (T2DM) (Kishimoto, Miyako, 2013). Metformin hydrochloride (MET) is 3-(diaminomethylidene)-1,1-dimethylguanidine;hydrochloride a oral antihyperglycemic drug. Metformin improves glucose tolerance in case of T2DM by decreasing hepatic glucose production, intestinal absorption of glucose, and by improving insulin sensitivity by increasing peripheral glucose uptake and utilization. Teneligliptin a peptidomimetic and metformin a biguanide in the combined dosage form is effective in the management of type 2 diabetes mellitus.

Literature survey reveals that many methods have been reported for the estimation of teneligliptin and metformin in pharmaceutical dosage form alone (Vishnu, Kiran *et al.*, 2016) or in combined dosage form using chromatographic methods (Deepak, Sufiyan *et al.*, 2017), (Manish, Mayank *et al.*, 2017). Further, it was observed that only one spectrophotometric method is reported for the simultaneous estimation of teneligliptin and metformin in the solid dosage form (Ashim, Denish *et al.*, 2016). Hence the present work describes a simple, precise and cost-effective UV-Spectrophotometric method for the determination of Teneligliptin hydrobromide hydrate and Metformin hydrochloride in bulk and tablet dosage form. The proposed methods were optimized and validated as per ICH guidelines.



(a) **(b)**

Figure I (a and b): Chemical structures of Teneligliptin and Metformin II. MATERIAL AND METHOD

2.1. Instrumentation

The entire study was performed on a double beam UV/VIS Spectrophotometer Cary 60 of Agilent Technologies. Also a Digital weighing balance TX 323L of Shimadzu Corporation and ultrasonicator of Sonar was used.

2.2. Chemical and Reagents

Teneligliptin hydrobromide hydrate (TEN) and Metformin HCl (MET) were supplied by Acme formulations and Cipla Ltd, India respectively as a gift sample. Double distillation water was used for study.

2.3. Preparation of Standard Stock Solution

Accurately about 50mg of each drug was weighed and transferred to 50ml of volumetric flask separately and dissolved in about 20ml of distill water. Both the solution were sonicated for 5 minutes. The volume was then made up to the mark with distill water. 10ml of each solution was then transferred to 100ml volumetric flask and diluted up to 100ml with distill water. These solution contained 100µg of drug per ml of the solution.

2.4. Determination of wavelength of maximum absorbance (λ_{max})

By appropriate dilution of standard stock solutions of teneligliptin and metformin in distill water, solutions containing 50µg/ml of teneligliptin and metformin were scanned separately in the UV region (200-400nm). The wavelength of maximum absorption was determined of both the drugs. Metformin showed maximum absorbance at 230nm and Teneligliptin at 245nm. An overlay spectrum of both the drugs showed its isobestic point at 240nm. Figure II

2.5. Method I – Simultaneous Equation Method (SE)

Different aliquots were taken from the stock solutions and diluted with distill water to prepare a series of concentrations of both drugs. The absorbances of these solutions were measured at 230nm and 245nm for Metformin and Teneligliptin respectively and calibration curves were plotted at selected wavelengths (figure III); the optical characteristics and linearity data is shown in table I. The E (1%, 1cm) of each drug at both wavelengths was determined shown in table II.

2.6. Method II- Q-Absorbance Ratio Method (AR)

The absorbances of the prepared solutions were measured at 230nm and 240nm for Metformin and Teneligliptin respectively and calibration curves were plotted at selected wavelengths (figure III); the optical characteristics and linearity data is shown in table I. The E (1%, 1cm) of each drug at both wavelengths was determined shown in table II.

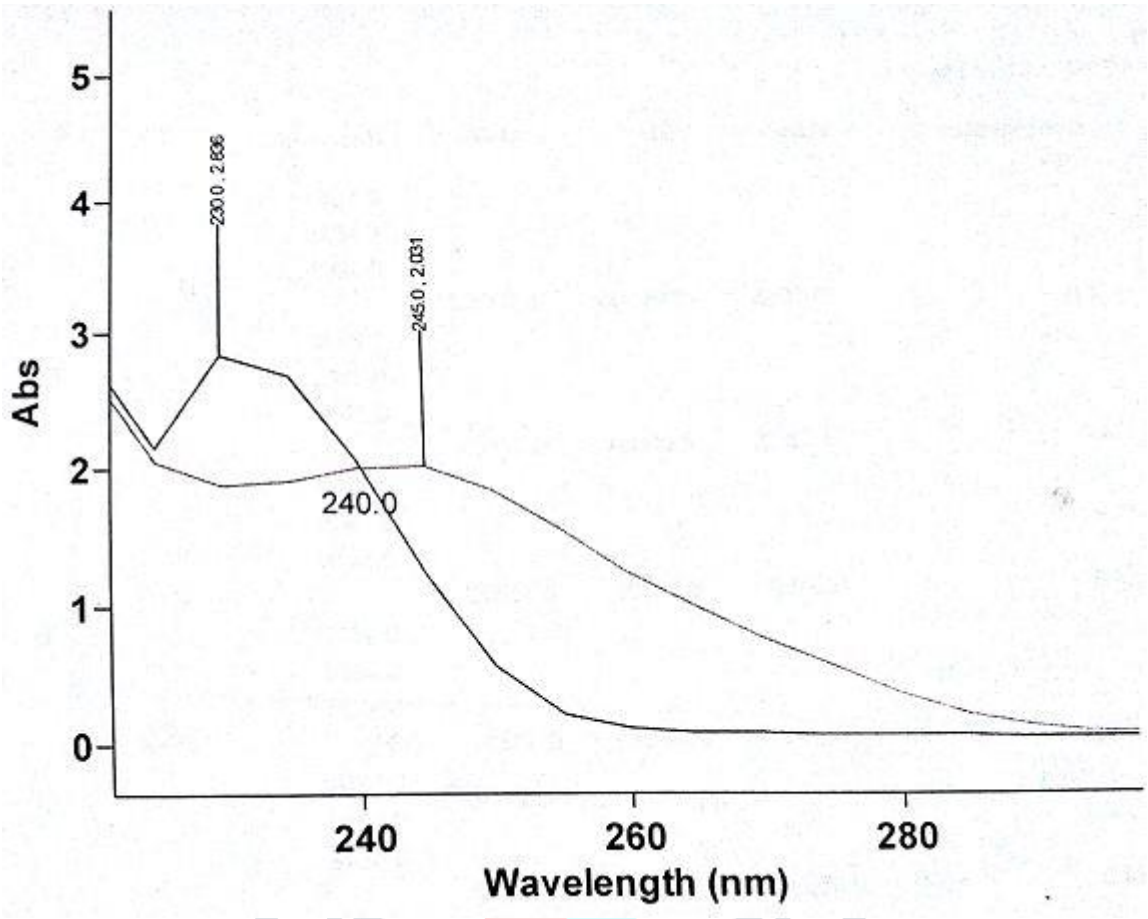
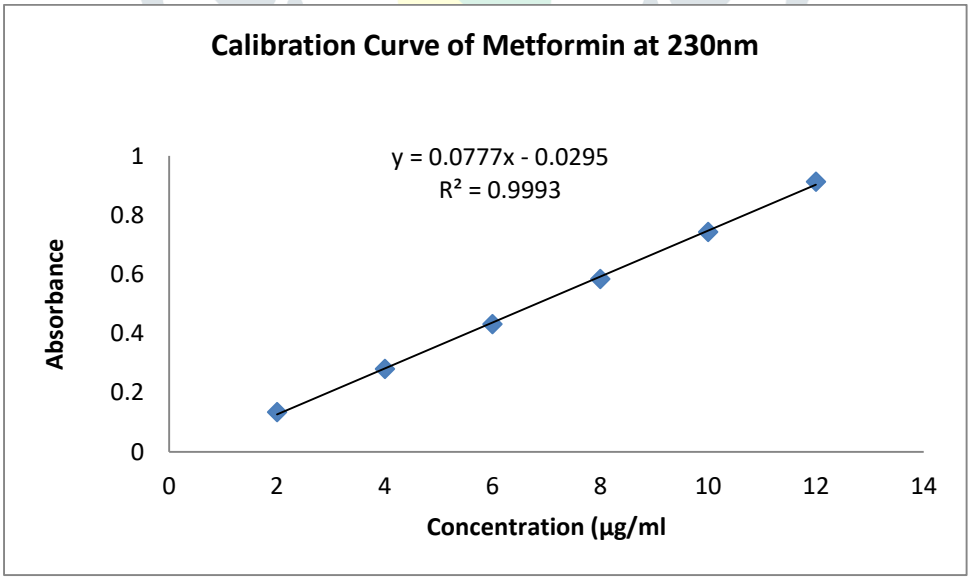
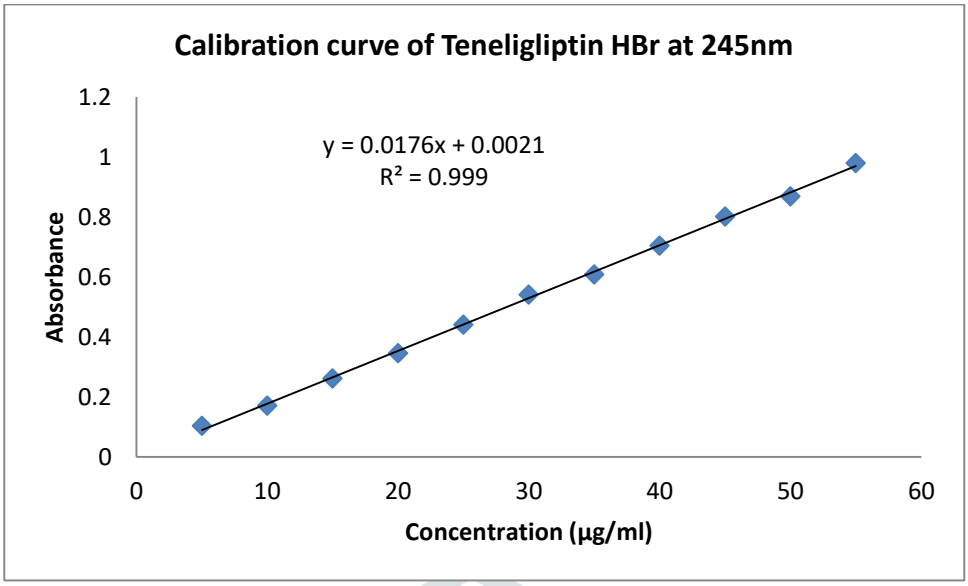


Figure II: Overlay spectrum of Teneligliptin and Metformin



(a)



(b)

Figure III a and b: Calibration curve of Metformin and Teneligliptin

Table I: Optical characteristics and linearity data

Parameters	METFORMIN	TENELIGLIPTIN
Beer's Law limit(µg/ml)	2-12	5-55
Molar absorptivity(1mole^{-1}cm^{-1})	117925	99522.090
Sandell's sensitivity (mg/cm^2/.001 absorbance unit)	14.04× 10^{-4}	46.78 × 10^{-4}
Regression equation (y= mx+c)	y = 0.077x - 0.029	y = 0.017x+0.002
Slope(m)	0.077	0.017
Intercept(c)	0.029	0.002
Correlation coefficient(R^2)	0.999	0.999

Table II: Absorptivity values for Method I (SE) and Method II (AR)

Drug	Method I (SE)		Method II (AR)	
	230nm	245nm	230nm	240nm
TEN	123	213.758	123	171.57
MET	712	354.82	712	564.965

***SE: Simultaneous equation; AR: Absorbance ratio; TEN: Teneligliptin; MET: Metformin**

2.7. Preparation and Analysis of Tablet solution

Contents of twenty tablets (containing 20mg of Teneligliptin and 500mg of Metformin) were weighed and ground to fine powder. For the analysis of tablets, a standard addition method was used. An accurately weighed 48mg of pure TEN was added to finely powdered sample to bring the concentration of Teneligliptin and Metformin as 1:1 in sample. A quantity of powdered tablets containing about 50mg of MET was weighed and transferred into 100 ml volumetric flask containing 40 ml of distill water, sonicated for 20 min, the volume was made upto the mark and filtered through Whatmann filter paper (No. 41). An appropriate volume of 10ml of this solution was transferred to 50 ml volumetric flask, dissolved and the volume was adjusted to mark. Appropriate aliquots were taken to prepare a solution containing 10µg per ml of drug. The absorbances of the solutions were measured at 230 nm and 245nm and 240nm against blank. The concentrations of two drugs in sample were determined by using method I and II. This procedure was performed on two marketed brands **'Afoglip'** and **'Dynaglipt'**. The results are reported in the Table III.

Table III: Analysis of Tablet formulations

Tablet Formulation		Label Claim	Amount Found (mg/tab)		% Label Claim		% RSD	
			Method SE	Method AR	Method SE	Method AR	Method SE	Method AR
AFOGLIP	TEN	20	19.84	19.42	99.24	97.08	1.14	1.02
	MET	500	493.3	494.45	98.66	98.89	0.39	0.6135
DYNAGLIPT	TEN	20	20.20	501.15	101.01	100.23	1.16	1.78
	MET	500	495.15	499.5	99.03	99.9	0.451	0.7971

***SE: Simultaneous equation; AR: Absorbance ratio; TEN: Teneligliptin; MET: Metformin**

2.8. Validation of Method

The method was validated as per the ICH guidelines 2005.

2.8.1. Linearity and Range

A linear relationship was observed between absorbance and concentration in the working range of 2-12µg/ml of Metformin and 5-55µg/ml of Teneligliptin of drug in the solution as shown in fig.III, and correlation coefficient (r) was as shown in table I.

2.8.2. Precision

Precision of the methods was studied as intra-day, interday. Intra-day study was performed by analyzing, a 10µg/ml concentration of drug for three times in the same day. Inter-day precision was performed by analyzing a 10µg/ml concentration of the drug for three days in a week. The results are shown in table IV.

Table IV: Precision study of TEN and MET

Drug	Actual Concentration (µg/ml)	Intraday precision				Interday precision			
		Method SE		Method AR		Method SE		Method AR	
		SD	%RSD	SD	%RSD	SD	%RSD	SD	%RSD
TEN	**10**	0.00049	0.334	0.0002	0.115	0.00010	0.052	0.00026	0.14
MET	**10**	0.00072	0.118	0.00080	0.103	0.0024	0.34	0.0021	0.33

***SE: Simultaneous equation; AR: Absorbance ratio; TEN: Teneligliptin; MET: Metformin**

2.8.3. Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of Metformin and Teneligliptin to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods; the results are shown in table V.

Table V: Recovery data of the proposed method

Drugs	Level %	Recovery %		% RSD	
		Method SE	Method AR	Method SE	Method AR
TEN	80	101.23	99.5	0.74	0.21
	100	101	97	1.15	1.37
	120	102.5	98.33	0.83	0.75
MET	80	98.75	102.5	0.769	0.98
	100	100	100.83	1.198	1.75
	120	99.16	98.9	0.601	1.18

***SE: Simultaneous equation; AR: Absorbance ratio; TEN: Teneligliptin; MET: Metformin**

III. RESULT AND DISCUSION

UV Spectrophotometric method namely simultaneous equation method and absorbance ratio method was developed and validated for the estimation of TEN and MET in the pharmaceutical dosage form. Two wavelengths, 230nm (Metformin) and 245nm (Teneligliptin) were selected for the simultaneous equation method. Whereas 230nm (Metformin) and 240nm (Teneligliptin) was selected for the estimation by absorption ratio method. The amount of drug estimated by the proposed method was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Both the methods were found to be precise as indicated by the inter-day, intra-day analysis, showing %RSD less than 2. All statistical data proves the validity of the methods and can be used for routine analysis of pharmaceutical formulations containing both these drugs.

IV. CONCLUSION

Simultaneous equation and absorbance ratio method was developed for the simultaneous estimation of TEN & MET in the combined tablet dosage form. It was observed that simultaneous equation method provide a better recovery results. The method developed was validated according to ICH guidelines. The method was found to be simple, precise, accurate and cost-effective. Moreover, all the developed UV-spectrophotometric methods require little sample preparation procedure with high sensitivity. Therefore, both the methods can be used successfully for routine quality control analysis of TEN and MET in combined tablet dosage form.

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REFERENCES

Al-Rimawi F. Development and validation of an analytical method for metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations by HPLC-UV. *Talanta.* 2009 Oct 15;79(5):1368-1371.

Arayne MS, Sultana N, Zuberi MH. Development and validation of RP-HPLC method for the analysis of metformin. *Pak J Pharm Sci.* 2006 Jul;19(3):231-235.

Arayne MS, Sultana N, Zuberi MV, Siddiqui FA. Spectrophotometric quantitation of metformin in bulk drug and pharmaceutical formulations using multivariate technique. *Indian journal of pharmaceutical sciences.* 2009 May;71(3):331.

Chitlange SS, Rawat DG, Chandani S. Estimation of Anti-diabetic Teneligliptin hydrobromide hydrate by RP-HPLC and Derivative Spectroscopy method. *Indo American Journal of Pharmaceutical Research.* 2016;6(7):6144-53.

Dhabale PN, Seervi CR. Simultaneous UV spectrophotometric method for estimation of gliclazide and metformine hydrochloride in tablet dosage form. *Int J Chem Tech Res*. 2010 Apr;2(2):813-7.

Drugbank [citad 2018 May 10]. Available from https://www.drugbank.ca/drugs/DB00331

Hemke, A., Rathod, E.A., Gupta, K.R., Umekar M. J. Hplc and uv-spectrophotometric estimation of teneligliptin from tablet dosage form. *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry.* 2016; 4(3):148-156.

Jeyabalan G, Nyola N. Simultaneous estimation of sitagliptin phosphate monohydrate and metformin hydrochloride in bulk and pharmaceutical formulation by RP-HPLC. *Journal of Pharmaceutical Education and Research.* 2012 Dec 1;3(2):24.

Kishimoto M. Teneligliptin: a DPP-4 inhibitor for the treatment of type 2 diabetes. Diabetes, metabolic syndrome and obesity: targets and therapy. 2013;6:187.

Kommineni V, Chowdary Kp, Prasad Sv. Development Of A New Stability Indicating Rp-Hplc Method For Simultaneous Estimation Of Saxagliptine And Dapagliflozin And Its Validation As Per Ich Guidelines. *Indo American Journal Of Pharmaceutical Sciences*. 2017 Sep 1;4(9):2920-32.

Kumar TG, Vidyadhara S, Narkhede NA, Silpa YS, Lakshmi MR. Method development, validation, and stability studies of teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy. *Journal of Analytical Science and Technology.* 2016 Dec 1;7(1):27.

Loni AB, Ghante MR, Sawant SD. Simultaneous UV spectrophotometric method for estimation of sitagliptin phosphate and metformin hydrochloride in bulk and tablet dosage form. *Der Pharma Chemica.* 2012;4(3):854-9.

Luhar SV, Pandya KR, Jani GK, Sachin B, Narkhed S. Simultaneous estimation of teneligliptin hydrobromide hydrate and its degradation product by RPHPLC method. *J Pharm Sci Bioscientific Res.* 2016;6(3):254-61.

Mali A.D., Mali S., Tamboli A., Bathe R. Simultaneous UV Spectrophotometric Methods for Estimation of Metformin HCl and Glimepride in Bulk and Tablet Dosage form. *International Journal of Advances in Pharmaceutics.* 2015 Dec; 4(6).

Mechanism of Action Pharmacokinetics- FDA. [citad 2018 May 15]. Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/021748s002lbl.pdf

Mishra K, Soni H, Nayak G, Patel SS, Singhai AK. Method development and validation of metformin hydrochloride in tablet dosage form. *Journal of Chemistry.* 2011;8(3):1309-13.

Patel A, Firke SD, Bari SB, Ranoliya JR. Development and Validation of Uv-Spectrophotometric Method for Simultaneous Estimation of Naproxen and Paracetamol By Q-Absorbance Ratio Method. *Int J Pharm Res Allied Sci*. 2014;3:57-63.

Patel BD, Ghate MD. Recent approaches to medicinal chemistry and therapeutic potential of dipeptidyl peptidase-4 (DPP-4) inhibitors. *European journal of medicinal chemistry*. 2014 Mar 3;74:574-605.

Patil, Deepak, et al. "Analytical method development and validation for the simultaneous estimation of Metformin and Teneligliptin by RP-HPLC in bulk and tablet dosage forms." *Journal of Pharmacy Research*. 2017 June; 11(6): 676-681.

Patil, M. D., et al. "Development and Validation of Analytical Method for Simultaneous Estimation of Metformin Hydrochloride and Teneligliptin Hydrobromide Hydrate in Pharmaceutical Dosage Form." *J Pharm Sci Bioscientific Res.* 2017; 7(2): 200-108.

Patil Sudarshan S, Bonde CG. Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV–visible spectroscopy. *International Journal of ChemTech Research*. 2009;1(4):905-9.

Pubchem Teneligliptin. [citad 2018 June 8]. Available from https://pubchem.ncbi.nlm.nih.gov/compound/Teneligliptin#section=Top

Sahoo PK, Sharma R, Chaturvedi SC. Simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride by RPHPLC method from combined tablet dosage form. *Indian journal of pharmaceutical sciences.* 2008 May;70(3):383.

Sen, Ashim Kumar. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods. *Journal Of Applied Pharmaceutical Sciences* 2016 Sep; 6 (09):157-165.

Sharma SK, Panneerselvam A, Singh KP, Parmar G, Gadge P, Swami OC. Teneligliptin in management of type 2 diabetes mellitus. Diabetes, metabolic syndrome and obesity: targets and therapy. 2016;9:251.

Shinde VC, Aher KB, Bhavar GB, Kakad SJ, Chaudhari SR. Development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation. *Der Pharmacia Lettre*. 2016;8(8):291-301.

Sonawane AM, Dhokale KK, Randhe VA. A simple uv-spectrophotometric method development and validation of teneligliptin in tablet dosage form. *Indo American Journal of Pharmaceutical Research.* 2016;6(04):5219-24.

Sunitha PG, Karthikeyan R, Ranjith Kumar B, Muniyappan S. Validated Colorimetric Methods for the Estimation of Teneligliptin in Tablets. *Journal of Drug Delivery and Therapeutics*. 2017 Jul 15;7(4):38-40.

Sujana K, Swathi Rani G, Bhanu Prasad M, Saheethi Reddy M, Sujana K. Simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride using UV spectroscopic method. *J Biomed Sci Res.* 2010;2(2):110-5.

WebMD Metormin HCl [citad 2018 May 25]. Available from https://www.webmd.com/drugs/2/drug-11285-7061/metformin-oral/metformin-oral/details

Yadav N, Goyal A. Method development and validation of Teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods. *International Journal of Pharmaceutical Chemistry and Analysis.* 2017;4(3):54-8.