Research Article

Simultaneous Equation Method Development and Validation for the Simultaneous Estimation of Teneligliptine hydrobromide hydrate (TEN) and Metformin hydrochloride (MET) in Tablet Dosage Form

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ABSTRACT

Precise and accurate UV spectroscopic methods were developed and validated for Teneligliptin hydrobromide hydrate (TENE) and Metformin hydrochloride (MET HCl) in pharmaceutical dosage form. Wavelengths selected for estimation were 243nm and 233nm in Simultaneous equation method and 233nm and 249.20 nm in absorbance ratio method for TENE and MET HCl respectively. Linearity of developed method was found to be 6-16µg/mL for TENE and MET HCl as the r2 value was found to be near 1. Accuracy was found to be 98.18%-102.81% in simultaneous estimation method and 98.33%- 101.11% in absorbance ratio method for TENE and MET HCl respectively. Method validated as per ICH guidelines.

Keywords: Simultaneous estimation, Teneligliptin hydrobromide hydrate and Metformin hydrochloride, UV Spectroscopic method, Validation.

INTRODUCTION

Diabetes is a group of metabolic disease in which a person suffers with high blood glucose (blood sugar), either because of low insulin production or because of body's cells inability to produce insulin properly, or both. A combination of Teneligliptin hydrobromide hydrate and Metformin hydrochloride producing synergistic effect in diabetes[1,5,6]. Teneligliptin hydrobromide hydrate is a Dipeptidyl peptidase 4 (DPP- 4) inhibitor. It is highly potent, competitive, and long lasting DPP-4 inhibitor[2]. Metformin hydrochloride is an antihyperglycemic agent which lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization[3].

MATERIALS AND METHODS

Teneligliptine hydrobromide hydrate was kindly gifted by Glenmark Pharmaceuticals Ltd., Mumbai, Metformin Hydrochloride procured from Norris Medicines Ltd., Ankleshwar, Methanol (HPLC Grade), Acetic acid (HPLC Grade) and Water (HPLC Grade) procured from Fischer scientific, Mumbai. Potassium dihydrogen phosphate procured from Merck, Mumbai

Method Development[7]

Selection of solvent

TENE and MET HCl were soluble in methanol and water. Hence water was selected for method development of both the drug as it was found that both the drugs were soluble in water.

Selection of wavelength

Standard drug solution of TENE and MET HCl were scanned separately in the range of 200-400 nm. Data was obtained by overlay spectra of TENE and MET HCl. Data was obtained as 233nm maxima wavelength of MET HCl and 243nm maxima wavelength of TENE for simultaneous estimation method.

Preparation of solutions

Preparation of stock solution for Metformin hydrochloride

An accurately weighed quantity of MET HCl (10mg) was transferred in to 100ml volumetric flask, 10-15ml of distilled water was added and sonicated for 5 min and diluted upto the mark 100ml with distilled water.

Preparation of stock solution for Teneligliptin hydrobromide hydrate

An accurately weighed quantity of TENE (10mg) was transferred in to 100ml volumetric flask, 10-15ml of distilled water was added and sonicated for 5 min and diluted upto the mark 100ml with distilled water.

Preparation of working standard solution for Metformin hydrochloride

From standard stock solution(100µg/ml) of MET HCl (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were taken and transferred in 10ml volumetric flask and make up the volume with distilled water, which gives (6, 8, 10, 12, 14, 16) µg/ml. Further absorbances of above prepared solutions were measured.

Preparation of working standard solution for Teneligliptin hydro bromide hydrate

From standard stock solution (100µg/ml) of TENE (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were taken and transferred in 10ml volumetric flask and make up the volume with distilled water, which gives (6, 8, 10, 12, 14, 16) µg/ml. Further absorbances of above prepared solutions were measured.

Method Validation[7, 8]

Linearity and Range

The linearity response was determined by analyzing 6 concentrations in the range of 6-16µg/ml for MET HCl and 6-16µg/ml for TENE. Accurately measured standard stock solutions of each MET HCl and TENE (0.6, 0.8, 1.0, 1.2, 1.4, 1.6) were transferred in to 10ml volumetric flask and make up the volume with distilled water to obtain concentration 6:6, 8:8, 10:10, 12:12, 14:14, 16:16µg/ml of MET HCl and TENE respectively. Absorbance of each solution was measured at 233 nm and 243nm.

Range in term which calibration curve constructed by plotting absorbance Vs concentration.

Precision

A) Repeatability

Repeatability of MET HCl and TENE checked by repeated measurement of absorbance of solution (n=6) of 10µg/ml (MET HCl) and 10µg/ml (TENE) measured and %RSD was calculated.

Acceptance criteria: - %RSD should be less than 2.

B) Intraday precision

Three replicates of three concentration of MET HCl (8, 10, 12 µg/ml) and TENE (8, 10, 12 µg/ml) total nine determinations were analyzed at same day within short time interval and absorbance were measured and % RSD was calculated.

Acceptance criteria:- %RSD should be less than 2.

C) Intermediate Precision

1. Interday Precision

Three replicates of three concentration of MET HCl (8, 10, 12 µg/ml) and TENE (8, 10, 12 µg/ml) total nine determinations were analyzed at three consecutive daysand absorbance were measured and % RSD was calculated.

Acceptance criteria:- %RSD should be less than 2.

2. Different instrument

Three concentration of MET HCl (8, 10, 12µg/ml) and TENE (8, 10, 12 µg/ml) total nine determinations were analyzed in two different instruments (UV-1800, UV-1700) on a same day and absorbance were measured and % RSD was calculated.

Acceptance criteria:- %RSD should be less than 2.

Robustness

Three different concentration of MET HCl (8, 10, 12 µg/ml) and TENE (8, 10, 12 µg/ml) were prepared and analyzed by different wavelength. The solution of MET HCl were analyzed at 232.5nm, 233nm, 233.5nm and TENE (8, 10, 12 µg/ml) were analyzed at 242.5nm, 243nm, 243.5nm. Absorbances at each wavelength were measured and % RSD was calculated.

Acceptance criteria:- %RSD should be less than 2.

Limit of Detection

The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

LOD=3.3(SD/Slope)

Where, SD = Standard deviation of the Y- intercepts of the 5 calibration curves

Slope= Mean slope of the 5 calibration curves.

Limit of Quantification

The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

LOQ= 10 (SD/Slope)

Where, SD= Standard deviation of the Y- intercepts of the 5 calibration curves

Slope= Mean slope of the 5 calibration curves

Accuracy

The accuracy of the method was determined by calculating %recovery of Metformin hydrochloride and Teneligliptine by standard addition method. Known amount of standard solutions of MET HCl and TENE corresponding to 50, 100 and 150% of target concentration were spiked with pre analyzed sample solution. The amounts of MET HCl and TENE were estimated by applying obtained values to regression equation of calibration curve.

Analysis of drug in marketed formulation[9]

The response of sample solution was measured at 233nm and 243nm. The amount of MET HCl and TENE present in sample solution were calculated.

Preparation of sample solution[4, 9]**:**

Take 20 tablets, average weight was calculated and powdered. Weigh equivalent to 25mg of Metformin hydrochloride and 1mg of Teneligliptin transferred in 100ml volumetric flask. 20ml of distilled water was added and sonicated for 15min. The volume was adjusted with distilled water up to the mark. The solution then filtered through whatman filter paper. To get final concentration 250µg/ml of Metformin hydrochloride and 10µg/ml of Teneligliptin. From this stock solution pipette out 2 ml and transferred in 10ml volumetric flask and make up volume up to the mark with distilled water to get concentration 2 µg/ml of TENE and 50 µg/ml of MET HCl. Then scanned in UV region and absorbance (A1) noted at 243nm and absorbance (A2) at 233 nm. At this point quantified TENE concentration.

From above sample solution pipette out 0.5ml and transferred in 10ml volumetric flask and make up volume up to mark with distilled water to get final concentration 0.5 µg/ml of TENE and 12.5 µg/ml of MET HCl.

Than scanned in UV region and absorbance noted (A1) at 243nm and absorbance (A2) at 233 nm and amount of MET HCl quantified.

RESULTS AND DISCUSSION

Selection of solvent

Water was selected for method development as both the drugs were found to be soluble in water.

Selection of wavelength

From the spectra of TENE the wavelength maxima selected for estimation at 243 nm and for MET HCl the wavelength maxima selected for estimation at 233 nm.

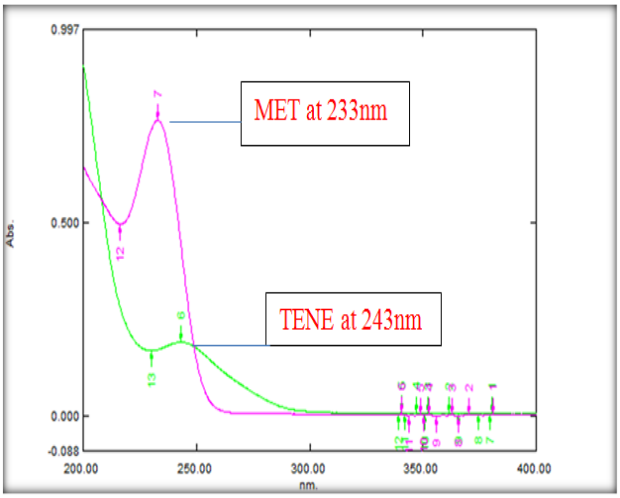

Figure 1: Overlay spectra of TENE and MET HCl (simultaneous equation method)

Validation of Simultaneous Equation Method

1) Linearity and Range

- Linearity study was carried out for both the drugs at different concentration levels. Linearity of TENE and MET HCl was in the range of 6-16 µg/ml and 6-16 µg/ml respectively.

Table 1: Data for linearity and range of TENE and MET HCl

Sr. No.	Concentration (µg/ml)		Absorbance at 243 nm		Absorbance at 233 nm	
	TENE	MET	TENE	MET	TENE	MET
1	6	6	0.109	0.277	0.097	0.464
2	8	8	0.152	0.369	0.134	0.620
3	10	10	0.191	0.452	0.173	0.763
4	12	12	0.234	0.533	0.213	0.900
5	14	14	0.268	0.632	0.239	1.066
6	16	16	0.309	0.715	0.275	1.207

2) Precision

A) Repeatability

The data for repeatability of absorbance measurement for TENE (10 µg/ml) and MET HCl (10 µg/ml) based on six measurement of same solution of TENE and MET HCl. The % RSD was found to be < 2.

Table 2: Repeatability data of TENE and MET HCl

Repeatability		
Concentration (10:10µg/ml)	**TENE at 243nm**	**MET HCl at 233nm**
1	0.191	0.779
2	0.192	0.760
3	0.187	0.763
4	0.187	0.780
5	0.189	0.773
6	0.191	0.763
Mean	0.189	0.769
SD	0.0021	0.0088
%RSD	1.1440	1.1435

B) Intraday Precision

It was performed by taking three replicates of standard solution of TENE and MET HCl using that 3 concentration (8, 10,12µg/ml) and (8, 10,12 µg/ml) were prepared thus total nine determination were analyzed within the short period of time interval. The % RSD was found to be < 2.

Table 3: Intraday Precision Data for TENE and MET HCl at 243nm

Drug	Concentration (µg/ml)	At 11am	At 2pm	At 5pm	Mean	SD	%RSD
TENE	8	0.152	0.154	0.150	0.152	0.0020	1.3157
	10	0.191	0.194	0.190	0.191	0.0020	1.0860
	12	0.234	0.229	0.234	0.232	0.0028	1.2425
MET HCl	8	0.369	0.366	0.370	0.368	0.0020	0.5651
	10	0.452	0.454	0.452	0.452	0.0011	0.2550
	12	0.533	0.546	0.546	0.541	0.0075	1.3856

Table 4: Intraday Precision Data for TENE and MET HCl at 233nm

Drug	Concentration (µg/ml)	At 11am	At 2pm	At 5pm	Mean	SD	%RSD
TENE	8	0.134	0.136	0.134	0.134	0.0011	0.8574
	10	0.173	0.171	0.169	0.171	0.0020	1.1695
	12	0.213	0.210	0.213	0.212	0.0017	0.8170
MET HCl	8	0.620	0.615	0.617	0.617	0.0025	0.4076
	10	0.763	0.760	0.763	0.762	0.0017	0.2273
	12	0.900	0.913	0.913	0.908	0.0075	0.8259

C) Intermediate Precision

1. Interday Precision

Three replicates of 3 concentrations of standard solution of TENE and MET HCl, total 9 determinations were analyzed at three consecutive day and absorbance were measured at 243 nm and 233 nm. % RSD was calculated. The % RSD was found to be < 2.

2. Different Instrument

Three different concentrations of TENE and MET HCl were analyzed at UV 1800 and UV1700 and record the absorbance at 243 nm and 233 nm. % RSD was calculated. The % RSD was found to be < 2.

3) Robustness

Robustness carried by changing wavelength ± 0.5 nm. %RSD for TENE and MET HCl was calculated. The % RSD was found to be < 2.

4) LOD and LOQ

Calibration curves were repeated for fiveand standard deviation of interceptwas calculated, then LOD and LOQ were calculated as follows:

Table 5: LOD and LOQ Data for TENE and MET HCl

Parameters	TENE at 243nm	MET HCl at 243nm	TENE at 233nm	MET HCl at 233nm
SD of the Y-Intercepts of 5 Calibration curve	0.0005	0.0005	0.0005	0.0004
Mean slope of 5 calibration curve	0.0182	0.0422	0.0162	0.0724
LOD(µg/ml)	0.0993	0.04283	0.1115	0.0203
LOQ(µg/ml)	0.3009	0.1297	0.3381	0.0617

5) Accuracy

From marketed formulation at three level of standard addition accuracy of the method was confirmed by recovery study. % recovery of TENE and MET HCl were found between 98% to 102%.

Table 6: % recovery data for TENE and MET HCl

Drug	% Level	Amt. of Sample taken (µg/ml)	Amt. of Standard Spiking (µg/ml)	Total Amt. (µg/ml)	Conc. Found (µg/ml)	%Recovery
TENE	I (50%)	6	3	9	8.90	98.88
		6	3	9	8.82	98.00
		6	3	9	8.99	99.88
TENE	II (100%)	6	6	12	11.90	99.16
		6	6	12	11.89	99.08
		6	6	12	11.94	99.50
TENE	III (150%)	6	9	15	15.10	101.33
		6	9	15	15.03	100.20
		6	9	15	14.93	99.53
MET HCl	I (50%)	6	3	9	9.15	101.66
		6	3	9	8.98	99.77
		6	3	9	8.90	98.88
MET HCl	II (100%)	6	6	12	12.02	100.20
		6	6	12	12.12	101.00
		6	6	12	12.21	101.75
MET HCl	III (150%)	6	9	15	14.72	98.19
		6	9	15	14.92	99.46
		6	9	15	14.87	99.13

6) Analysis of Marketed Formulation

Table 7: Analysis of TENIVA-M Tablet

Sample	Label Claim%		Amt. Found		%Assay	
	TENE (mg/tab)	MET HCl (mg/tab)	TENE (mg/tab)	MET HCl (mg/tab)	TENE	MET HCl
1	20	500	19.60	495.20	98	99.04
2	20	500	19.74	495.50	98.73	99.10
3	20	500	19.86	494.15	99.32	98.83
4	20	500	19.99	496.6	99.95	99.32
5	20	500	19.72	498.9	98.60	99.78
6	20	500	20.04	497	100.20	99.40
Average			**19.82**	**496.22**	**594.8**	**99.24**

DISCUSSION

Simultaneous Equation Method was developed and validated for Teneligliptine and Metformin hydrochloride. Linearity was found near to 1, for Teneligliptine and Metformin hydrochloride. For Intraday, Interday, Intermediate precision, Robustness, % RSD was found less than 2. % Recovery was found to be between range 98-102% for both the drugs. These results indicate that the method is accurate, precise and simple.

CONCLUSIONS

Both the drugs showed better solubility and stability in Methanol. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed method is new, simple,

accurate, sensitive, economic and precise and can be adopted for routine analysis and in tablet dosage form.

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REFERENCES

1. Medicalnewstoday.com [homepage on the Internet]. United Kingdom: Diabetes: Symptoms, Causes and Treatments [updated 2002 May 16; cited 2017 November]. Available from: http://www.medicalnewstoday.com/info/diabetes.
2. Pharmacodia.com [homepage on the Internet]. Beijing: Teneligliptin Hydrobromide Hydrate [updated 2016 May 17; cited 2017 November]. Available from: https://www.pharmacodia.com/yaodu/html/v1/chemicals/186fb23a33995d91ce3c2212189178c8.html
3. Pharmacodia.com [homepage on the Internet]. Beijing: Metformin Hydrochloride [updated 2016 August 25; cited 2017 November]. Available from: http://en.pharmacodia.com/web/drug/1_6864.html
4. Metformin Hydrochloride: Indian Pharmacopoeia, Government of India, Ministry of Health & Family Welfare: Publisher: The Indian Pharmacopoeia Commission: Ghaziabad; 2010. Volume II. p. 1657-1658.
5. Tripathi K D: Essentials of medical pharmacology: 6th edition. India: Jaypee publishers; 2010, pp: 254-255.
6. Cornwall Jon. Current states of diabetes mellitus in India. Australasian Medical Journal: 7(1), 2014, pp: 45-48.
7. ICH Q2 (R1), VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY, Current Step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005)1996. Available from: http://www.ich.org/fileadmin/Public _Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
8. Indian Pharmacopoeia, Government of India, Ministry of Health & Family Welfare: Publisher: The Indian Pharmacopoeia Commission: Ghaziabad; Volume I, 2010. p. 198.
9. Medplusmart.com [homepage on the Internet]. Drug information on "Metformin hydrochloride and Teneligliptin" [updated 2016 June 11; cited 2017 November] https://www.medplusmart.com/searchAll/dGVuZXZh
10. Luhar S, Pandya K, Jani G, Narkhed S. Simultaneous Estimation of Teneligliptin Hydrobromide Hydrate and its Degradation Product by RP-HPLC Method. J Pharm Sci Bioscientific Res [Internet]. 6(3), 2016 Aug [cited 2016 Aug 3], [about 8pp.]. Available from http://www.jpsbr.org/volume_6/JPSBR_Vol_6_Issue_1_htm_files
11. Shinde V, Aher K, Bhavar G, Kakad S, Chaudhari S. 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 [Internet]. 8(8), 2016 Aug [cited 2016 Aug 5], [about 10pp.]. Available from http://scholarsresearchlibrary.com/dpl-vol8-iss8/DPL-2016-8-8
12. Chunduri R, Dannana G. Development And Validation Of LC-MS/MS Method For Quantification Of Teneligliptin In Human Plasma And Its Application To A Pharmacokinetic Study. World Journal of Pharmacy And Pharmaceutical Sciences [Internet]. 5(5), 2016 Aug [cited 2016 Aug 10], [about 12 pp.]
13. Ganesh Kumar T, Vidyadhara S, Narkhede A, Sai S, Rajya L, 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. [Internet]. 2016 Aug [cited 2016 Aug 10]; [about 8pp.] Available from http://link.springer.com/article/10.1186/s40543-016-0099-0
14. Chitlange S, Rawat D, Chandani S. Estimation Of Anti-diabetic Teneligliptin Hydrobromide Hydrate By Rp-hplc And Derivative Spectroscopic Method.Indo american journal of pharmaceutical research. [Internet]. 6(7), 2016 Aug [cited 2016 Aug 12], [about 10pp.] Available from: http://www.iajpr.com/archive/volume-6/july-2016/16july20.html
15. Sonawane A, Dhokale K, Randhe V.A Simple UV-Spectrophotometric Method Development and Validation Of Teneligliptin In Tablet Dosage Form. [Internet]. 6(4), 2016 Aug [cited 2016 Aug 12]; [about 6pp.] Available from http://www.scopemed.org.
16. Madhukar A, Prince A, Vijay Kumar R, Sanjeeva Y, Jagadeeshwar K, Raghupratap D. Simple And Sensitive Analytical Method Development And Validation Of Metformin Hydrochloride By Rp-hplc. Int. J. Pharm Pharm Sci [Internet]. 3(3), 2016 Aug [cited 2016 Aug 17], [about 4pp.] Available from http://www.ijppsjournal.com/Vol3Issue3/2202
17. Chhetri H, Thapa P, Schepdael V. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharmaceutical Journal. [Internet]. (22), 2016 Aug [cited 2016 Aug 17]; [about 4pp.] Available from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4246406/
18. Patil S, Bonde C. Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets. Int.J. ChemTech. [Internet]. 1(4), 2009 oct-dec [cited 2016 Aug 20]; [about 5pp.] Available from http://sphinxsai.com/CTVOL4/ct_pdf_vol_4/CT=16%20(905-909).pdf
19. Loni A, Ghante M, Sawant S. Simultaneous UV spectrophotometric method for estimation of sitagliptin phosphate and metformin hydrochloride in bulk and tablet dosage form. Scholar research library. [Internet] 4(3), 2012 [cited Aug 20]; [about 6pp.] available from http://derpharmachemica.com/archive.html
20. Jajow S, Chandaka M, Mallepelli S. Analytical Method Development and Method Validation for the Simultaneous Estimation of Metformin hydrochloride and Pioglitazone hydrochloride in Tablet Dosage Form by RP-HPLC. Asian J. Pharm; Ana. [Internet] 2(3), 2012 [cited Aug 24], [about 5pp.] Available from www.asianpharmaonline.org

21. K Neelima, Prasad R. Analytical Method Development and Validation of Metformin,Voglibose, Glimepiride in Bulk and Combined Tablet Dosage Form by Gradient RP-HPLC. Pharmaceutical Methods. [Internet] 5(1), 2014 Jan-Jun [cited Aug 24]; [about 7pp.] Available from

http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.683.9438&rep=rep1&type=pdf

22. Bonde S, Gaikwadb A, Kataleb D, Gavalib S. A Simple And Sensitive Method For Determination Of Metformin And Sitagliptin In Human Plasma Using Liquid Chromatography And Tandem Mass Spectrometry. Int J Pharm Pharm Sci. [Internet] 5(3), 2013 Jun [cited 2016 Aug 26]; [about 8pp.] Available from

http://www.ijppsjournal.com/Vol5Suppl3/7371.pdf

23. Pandya R, Rathod R, Maheswari D. Bioanalytical Method Development and Validation for Simultaneous Determination of Linagliptin and Metformin Drugs in Human Plasma By Rp-hplc Method. Pharmacophore. [Internet] 5(2), 2014 [cited september 2], [about 8pp.]Available from

http://www.pharmacophorejournal.com/issue/Pharmacophore_ March-April_2014_article1.pdf

24. Ashim Kumar Sen* , Denish N. Hinsu, Dhanya B. Sen, Aarti S. Zanwar, Rajesh A. Maheshwari, Vikas R. Chandrakar Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from its pharmaceutical dosage form by three different UV spectrophotometric methods. Journal of Applied Pharmaceutical Science: September, Vol. 6 (09), 2016, pp. 157-165

25. Avani P. Khristi, Dr.R.B.Mardia, Dr. B.N.Suhagia. "UV Spectrophotometric Method Development and Validation of First Derivative Method for the simultaneous estimation of Sildenafil Citrate (SIL) and Aspirin (ASP) in bulk and tablet dosage form. (Indo American Journal of Pharmaceutical Research, 5(09), 2015, 2837-2843

26. Manisha Kotadiya*, Avani Khristi, Quantitative Determination and Validation of Teneligliptine Hydrobromide Hydrate Using FTIR Spectroscopy (Journal of Chemical and Pharmaceutical Research, 9(11), 2017, 109-114)

27. Hardi Joshi, Avani Khristi, "UV Spectrophotometric Method Development and Validation of Absorbance ratio method for the simultaneous estimation of Teneligliptine (TEN) and Metformin Hydrochloride (MET) in tablet dosage form. (International Research Journal of Pharmacy, 2018, 9(1), 47-55)Ewing GW. Instrumental methods of chemical analysis: 5th ed. New York: Mc Graw – Hill book company, 1985, p.1-7.

28. Mendham J., Denny RC. and Baraes JD. Vogels Text book of Quantitative chemical analysis: 5th ed. Singapore: Pears Education, 1985, p.5-11.

29. Snyder LR., Kirkland JJ. Introduction to Modern Liquid Chromatography: 4th ed. New York: John Wiley & Sons; Inc, 1997, p. 267-400.

30. Skoog BK., West DM., and Hollar DF. Fundamentals of Analytical Chemistry: 8th ed. Meerut: Saunders Collage Publishing, 2004, p.1-6.

31. Chatwal GR., Anand SK. Instrumental Methods of Chemical Analysis, 5th ed.Himalaya Publishing House, p.2.177, 2.78, 2.340, 2.185, 2.272.

32. Skoog DA., Holler FJ., and Nieman TA. An Introduction to Analytical Chemistry: 5th ed. Singapore: Thomson Books/Cole Publication, 1994, p.566-568.

33. Sharma BK. Instrumental Methods of Chemical Analysis: 23rd ed. Meerut: GOEL Publication House, 2004, p.114-165,285-320.

34. Backett AH, and Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi:CBS Publications and Distributors; Part-II, 2001, p. 285-297.

35. Sethi PD., High performance liquid chromatography Quantitative analysis of pharmaceutical formulations: New Delhi: CBS publishers, 2001, p. 101-135.

36. Ahuja S., Dong WM. Handbook of pharmaceutical analysis by HPLC. Canada:Separation science technology; Vol.6, p. 48.

37. Kasture AV., Wadodkar SG., Mahadik KR., More HM. In introduction to instrumental Techniues. 12th ed. Pune: Nirali Prakashan, 2002, p. 1-3.

38. Patel GC and Jani GK. Basic Biostatistics for Pharmacy. 15th ed. Atul Prakashan, 2006, p.183-184.

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