Simultaneous Estimation of Linagliptin and Metformin Hydrochloride in Bulk and Combined Tablet Dosage Form by UV-Spectrophotometric Methods

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ABSTRACT

Two simple, accurate, rapid and precise UV Spectrophotometric methods have been developed for simultaneous estimation of Linagliptin (LIN) and Metformin hydrochloride (MET) in combined tablet dosage form. The methods employed were (A) Simultaneous equation and (B) Second order derivative method. Method-A involves which involved measuring the absorbance values at 297 nmand 232nmof overlay spectrum of LIN &MET respectively. Method B is Second order derivative spectrophotometry, which involved measuring the absorbance values at 297 nm and 232nm of second derivative spectrum of LIN &MET respectively. In both the methods linearity was found in the concentration range of 0.5-2.5 µg/ml and 5-25 µg/ml respectively. Both the methods were found to be rapid, specific, precise and accurate. Hence these methods can be applied for routine analysis of Linagliptin and Metformin hydrochloride in combined dosage form without any interference by the excipients. The above methods are validated according to ICH guidelines.

Keywords: Linagliptin, Metformin hydrochloride, Simultaneous equation method, Second order derivative method

INTRODUCTION:

LINAGLIPTIN is an orally administered anti-diabetic drug in the DPP-4 inhibitor class. LINAGLIPTIN inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1). The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control. Only one liquid chromatography method has been reported for the estimation of LINAGLIPTIN individually. No other methods are available for individual drug and in any combinations.

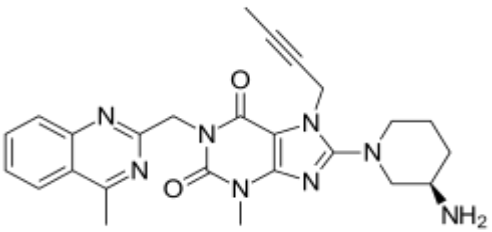


Figure 1: Chemical structure of LIN:

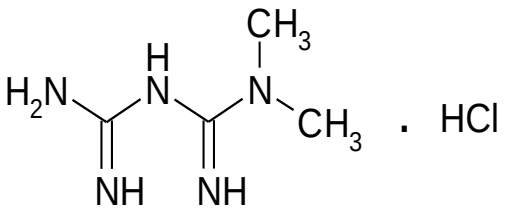


Figure 2: Chemical structure of MET

Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus [NIDDM]. It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. There are very few reports on analytical methods for estimation of Metformin individually and in other combinations, which includes bio analytical methods using rat plasma, RP-HPLC, Spectrophotometric methods. The combination of Linagliptin and Metformin was significantly superior with proper diet and high blood sugar. On literature survey, it was found that no method has been reported for simultaneous estimation of Linagliptin and Metformin in combined dosage form and no method is available in pharmacopeia. Hence an attempt has been made to develop a simple, accurate, precise and reproducible Q absorbance ratio and area under curve methods for simultaneous estimation of LIN and MET in combined dosage form and the same has been validated as per recommended ICH guidelines.

EXPERIMENTAL:

Apparatus:

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measureabsorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. An Electronic balance of capacity 220g SHIMADZU COOPRATION JAPAN.

Preparation of standard stock solutions:

100 mg each of Linagliptin and Metformin hydrochloride were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of solvent by ultrasonication and then volume was made up to the mark with Solvent to obtain concentration of 1000 µg/ml of each component (stock A and A' solution). From the above stock A and A' solution 10 ml of aliquot was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with solvent to obtain the final concentration of 100µg/ml of each component (stock B and B' solution).

Preparation of sample stock solution using formulation:

Twenty tablets of Linagliptin and Metformin hydrochloride (ONDERO MET) in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 25 mg of Linagliptin was weighed which also contains 250 mg of Metformin hydrochloride and transferred to 100 ml volumetric flask and dissolved in 50 ml solvent and the content was kept in ultrasonicator for 15 min. The solution was filtered through Whatmann filter paper No.41, finally the volume was made up to the mark with solvent, which gave a concentration of 250µg/ml of Linagliptin and 2500µg/ml of Metformin hydrochloride and this solution was used as stock 'A' solution. From the above stock 'A' solution, 5 ml of the aliquot was pipetted out and was transferred to a 50 ml volumetric flask. The volume was made up to 50 ml with solvent to obtain a solution with final concentration of 25µg/ml Linagliptin and 250µg/ml of Metformin hydrochloride (stock B).

METHODS:

Method A: Simultaneous Equation Method:

From the standard stockB and B'solutions, dilutions ranging between1-90µg/mL for both the drugs were prepared and scanned in the wavelength range of 400-200nm using UV- visible spectrophotometer. At297 nm Linagliptin showed maximum absorbance andat 232nm Metformin hydrochloride shows maximum absorbance. Both the drugs did not show any interference at either of the wavelength. Hence 297 nm and 232 nm for Linagliptin and Metformin hydrochloride were selected as the working analytical wavelength.

Method B: Second Order Derivative Method:

From the standard stock B and B' solutions, dilutions ranging between 0.5 to 2.5µg/ml of Linagliptin and 5 to 25µg/mL of Metformin hydrochloride were prepared and scanned in the wavelength range of 400-200 nm using UV Spectrophotometer. The absorbance spectrum, thus obtained were derivatized to remove the interference of absorbing species. From the examination of the second order derivative spectrum of Linagliptin and Metformin Hydrochloride, 297nm (λ_1) and 232nm (λ_2) were selected as working wavelengths for the second order derivative spectroscopy.

Validation of the methods:
All the methods were validated according to ICH guidelines by carrying out analysis of six replicate samples of tablet. Recovery studies were carried out at three different levels i.e., 80%, 100% and 120% by adding the pure drug to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated.

RESULTS AND DISCUSSION:

The estimation of Linagliptin and Metformin hydrochloride in tablet formulation was found to be accurate and reproducible with a linearity of 0.5 to 2.5µg/mL and 5 to 25µg/mL respectively for both the methods and the correlation coefficient 0.998 and 0.999 for method A and 0.998 and 0.998 for method B. The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in Table 1 and Table 2 for method A and method B respectively. Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (r) were calculated and are presented in Table 1 and Table 2 for method A and method B respectively.The accuracy was found by recovery studies at three different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, an indicative of the accuracy of the methods.
The results of Formulation were reported in Table-3. The spectra of Linagliptin, Metformin hydrochlorideand formulation are reported bySimultaneous Estimation method (Fig. 3, 4 and 5) and calibration curve was plotted (Fig. 6, 7, 8 and 9).

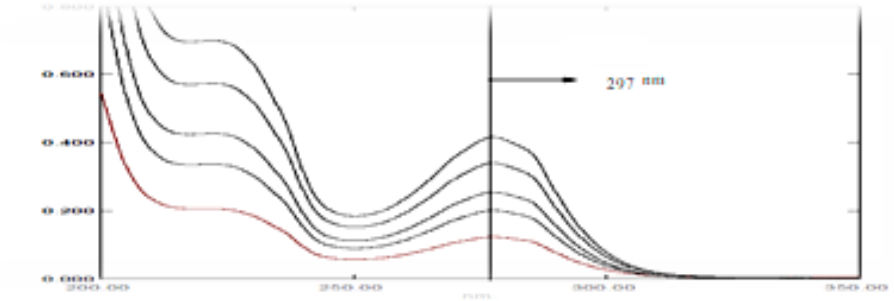


Fig.3: Overlay Spectrum of Linagliptin at 297 nm in Distilled water.

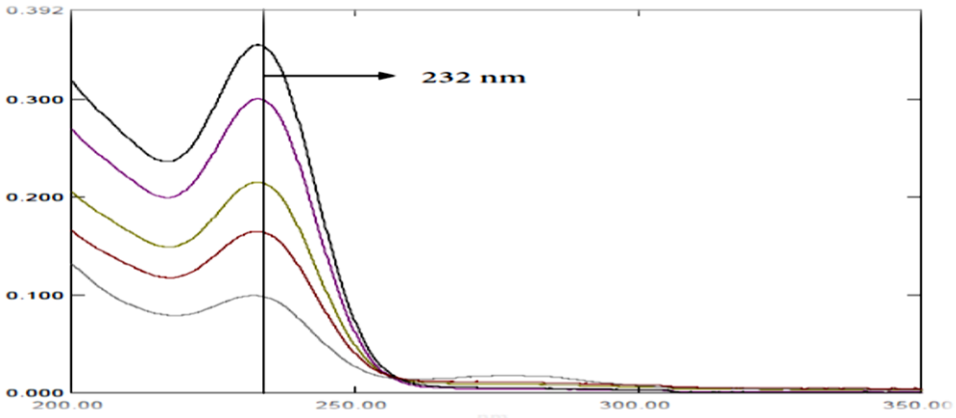


Fig.4: Overlay Spectra of Metformin hydrochloride at 232 nm in Distilled water

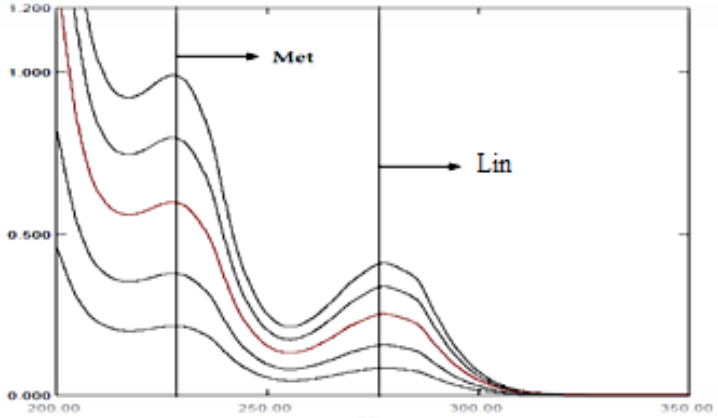
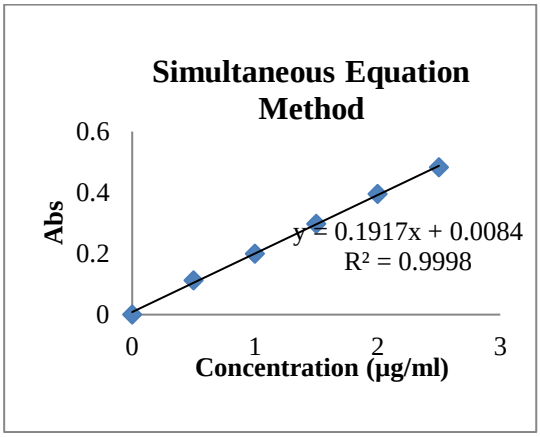


Fig.5: OverlaySpectra of Formulation in Distilled water in Distilled water.



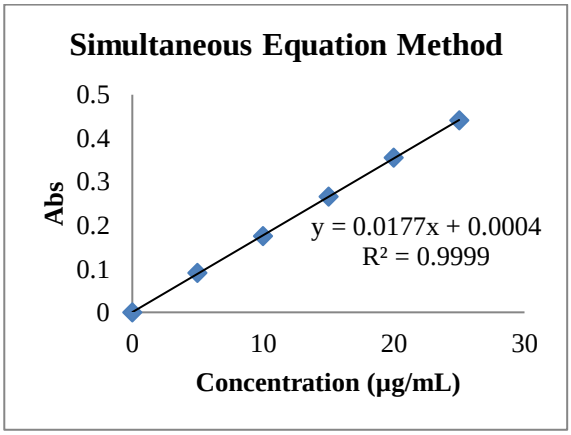
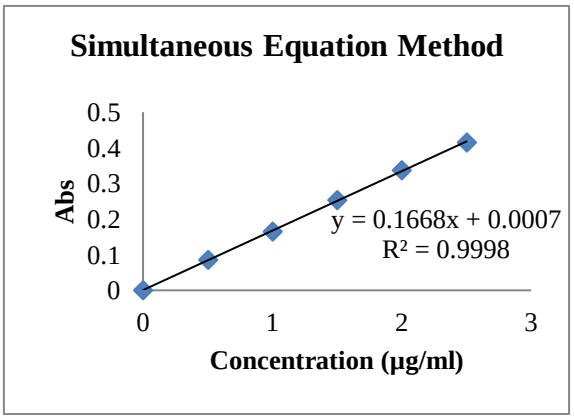


Fig. 6& 7: Calibrationcurve for Linagliptin at 297 nmand Metformin hydrochloride at 232 nm by Simultaneous Equation Method



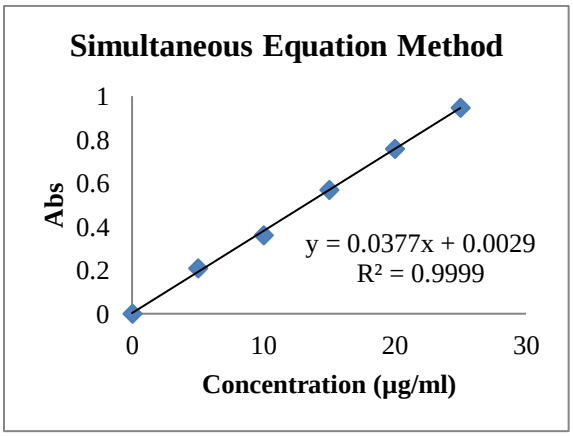


Fig.8& 9: Calibration curve for LIN in Formulation at 297 nm &MET in Formulation at 232 nmbySimultaneous Equation Method

The Second order derivative spectrum of LIN at 297 nm, MET at 232 nm and formulationare reported (Fig.10,11,12) and calibration curve was plotted (Fig.13,14, 15,16).

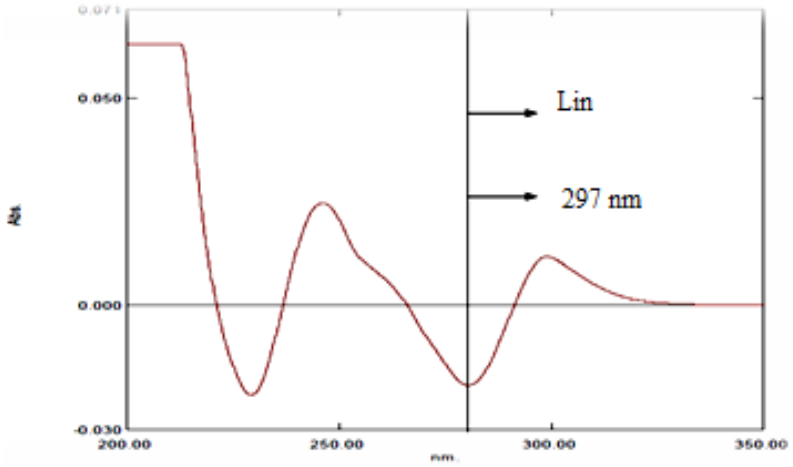


Fig. 10: Second order derivative spectrum of LIN at 297 nm.

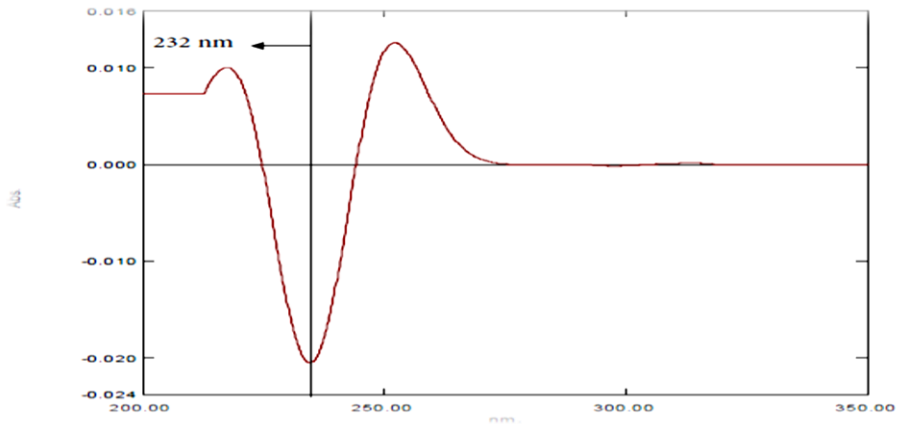


Fig. 11: Second order derivative spectrum of MET at 232 nm.

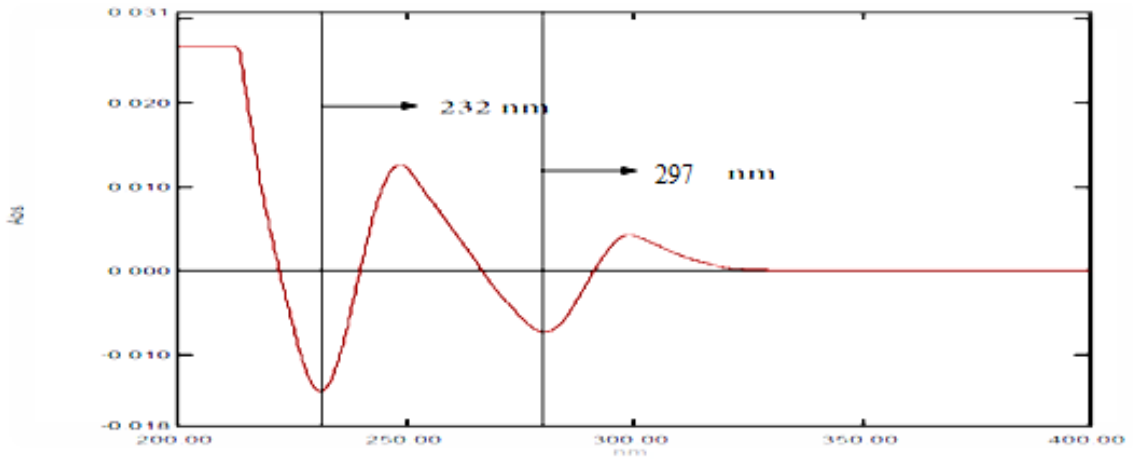
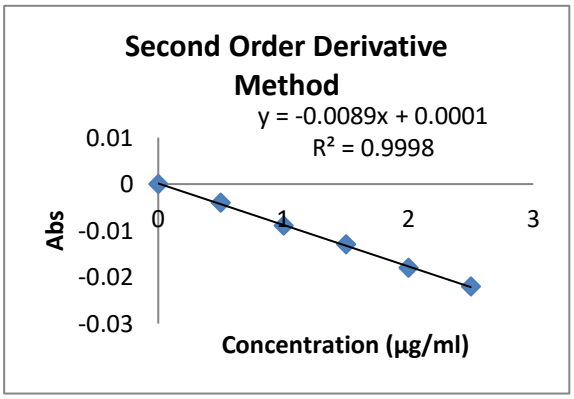


Fig 12: Second order derivative spectrum of LIN and MET in Formulation at 232 and 297 nm



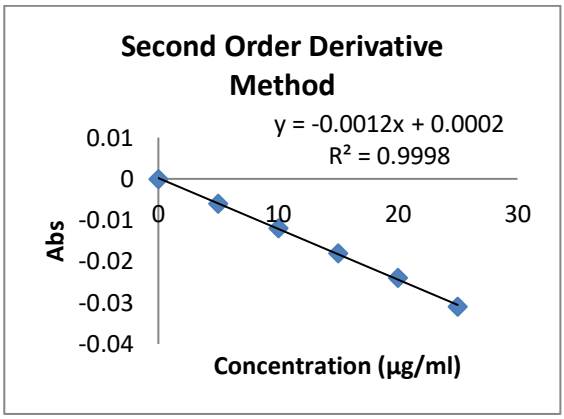
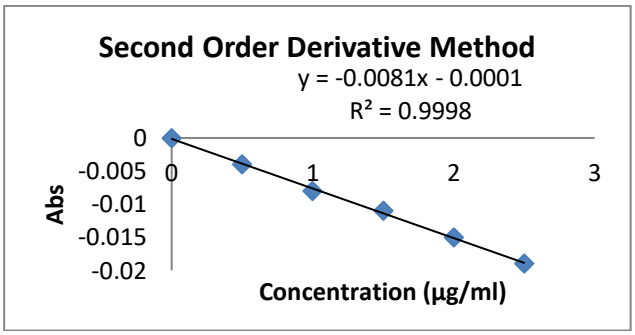


Fig. 13 & 14: Calibration curve for LIN at 297nm and MET at 232 nm by Second Order Derivative Method



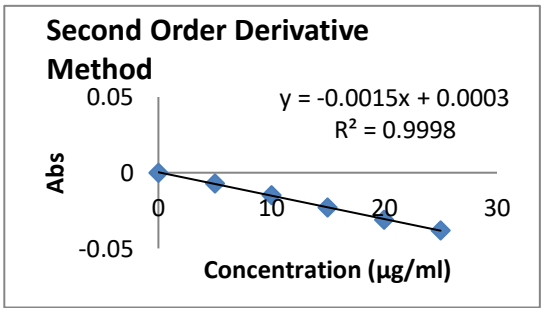


Fig. 15& 16: Calibration curve for LIN in Formulation at 297 nm & MET in Formulation at 232 nm by Second Order derivative Method

Table 1:OpticalcharacteristicsandotherparametersforMethodA

Parameters		LIN	MET
Linear range (µg/ml)		0.5 - 2.5	5 - 25
λ_{max}/wavelengthrange(nm)		**297 nm**	**232 nm**
Coefficient of correlation		0.9998	0.9999
Slope*(m)		0.1917	0.017
Intercept*(c)		0.0084	0.0004
Accuracy (%RSD)	80%	0.0472	0.02081
	100%	0.0611	0.01527
	120%	0.1201	0.01
Precision (%RSD)	Intra-day	0.1720	0.5091
	Inter-day	0.0159	0.0001
Limit of Detection (µg/ml)		0.014	0.043
Limit of Quantification (µg/ml)		0.229	0.699

*y=mx+c;whenxistheconcentrationinµg/mlandyisabsorbanceunit.

Table 2: Optical characteristics and other parameters for MethodB

Parameters		LIN	MET
Linear range (µg/ml)		0.5 - 2.5	5 - 25
λ_{max}/wavelengthrange(nm)		**297 nm**	**232 nm**
Coefficient of correlation		0.9998	0.9999
Slope*(m)		-0.0089	-0.0012
Intercept*(c)		0.0001	0.0002
Accuracy (%RSD)	80%	0.0472	0.02081
	100%	0.0611	0.01527
	120%	0.1201	0.01
Precision (%RSD)	Intra-day	0.079	0.1715
	Inter-day	0.04	0.01
Limit of Detection (µg/ml)		0.207	0.297
Limit of Quantification (µg/ml)		0.63	0.841

*y=mx+c;whenxistheconcentrationinµg/mlandyisabsorbanceunit.

Table 3:Resultsofformulation

method	Brand name	Label claim of LIN (mg)	Label claim of MET (mg)	Amount found for LIN (mg)	Amount found for MET (mg)	%Recovery ±SD** for LIN	%Recovery ±SD** for MET
A	ONDERO MET	25	250	25.015	249.863	99.84±0.42780	99.95±0.01505
B		25	250	25.005	249.888	100.04±0.2245	99.96±0.02345

** Average of six determinations

CONCLUSION:

All these factors leads to conclusion that proposed absorption correction method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of LIN and MET as compare to the developed simultaneous equation by matrix. The developed method was validated as per ICH guidelines. Statistical analysis proved that the methods is repeatable and selective for the analysis of LIN and MET in their combined pharmaceutical formulations.

REFERENCES:

Amar Gangadhar Zalte, Ravindranath Bhanudas Saudagar, Pramod Nanasaheb Katkade, (2016). Validated UV spectroscopic estimation of Linagliptin concentration in bulk and dosage forms. Volume - 9, Issue.

Amruta B L, Minal R G, Sawant S D. (2012). Simultaneous UV spectrophotometric method for estimation of Sitagliptin Phosphate and Metformin Hydrochloride in bulk and tablet dosage form. Der Pharma Chemical. 4(3): 854-9.

Dhabale P N, Seervi C R. (2010). Simultaneous UV Spectrophotometric Method for Estimation of Gliclazide and Metformine Hydrochloride in tablet dosage form. CODEN (USA) *IJCRGG*. 2(2):813-7.

John H B, John M B. (2004). Wilson & Gisvold's textbook of Organic medicinal and Pharmaceutical Chemistry. 11th ed. p. 672.

Laurene L B, Jhon S L, Keith L P. (2006). *Goodman and Gilman's the pharmacological basis of Therapeutics*. 11th ed. Mc Graw Hill, New York, U.S.A. p. 1638-39.

Maria C R, Mihaela I, Lavinia H, Elena I, Valentina A, Aurelian E R. et al. (2009). Constantin M. Validation of a HPLC method for the simultaneous analysis of Metformin and Gliclazide in human plasma. *FARMACIA*. 57(6):728-35.

Ramzia I E, Ehab F E, Bassam M A. (September, 2012). Liquid Chromatographic determination of Alogliptin in bulk and in its pharmaceutical preparation. *Int J Biomed Sc*,8(3):215-8.

Sujana K, Swathi R G, Bhanu P M, Saheethi R M. (2010). Simultaneous estimation of Pioglitazone Hydrochloride and Metformin Hydrochloride using UV spectroscopic method in tablet dosage form. *J Biomed Sci & Res.* 2(2):110-5.

Wanjari M M, There A W, Tajne M R, Chopde C T, (Mar-Apr2008). Umathe S. N. Rapid and simple RPHPLC method for the estimation of Metformin in rat plasma. *Indian J Pharm Sci.*70(2):198-202.
