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Development and validation of UV-Visible spectroscopy method for simultaneous estimation of Saxagliptin hydrochloride and metformin hydrochloride in tablet dosage form

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Abstract
A simple, rapid and validated analytical method has been developed for estimation of Saxagliptin and Metformin in tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) of Saxagliptin were found to be 274nm and Metformin 231nm. The percentage recovery of Saxagliptin(API) 100.10% and Metformin(API) 99.98%. Beer's laws were obeyed in the concentration range 50-90 µg/ml for Saxagliptin and 2-10µg/ml for Metformin. The linear equation of Saxagliptin and Metformin was calculated. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantitation. Validation was performed as per ICH guidelines. The proposed method was successfully applied for the quantitative determination of Metformin and Saxagliptin in tablet dosage form (percentage label claim 99.60% and 100% respectively).

Keywords: Saxagliptin, metformin, simultaneous estimation

Introduction

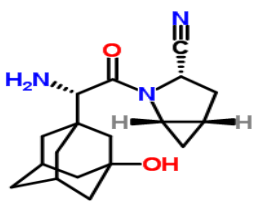
Gliptin

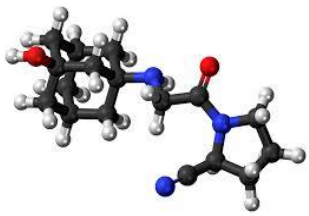
Dipeptidyl peptidase-4 inhibitor (DPP-4) they are prescribed to type-2 Diabetes patients. DPP-4 inhibitor may help with weight loss as well as decrease blood glucose level.
Derivatives of Gliptins are

a. Alogliptin
b. Linagliptin
c. Saxagliptin
d. Sitagliptin.

Saxagliptin

This is new oral hypoglycemic of the new DPP-4 inhibitor class of drugs. Saxagliptin is chemically identify as (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3 Hydroxytricyclo[3.3.1.13,7]dec-1-yl)acetyl]-2 azabicyclo[3.1.0]hexane-3-carbonitrile previously identified as BMS-477118.
Empirical formula is $C_{18}H_{25}N_3O_2$, H_2O and molecular weight 333.43. It is sparingly soluble in water and soluble in methanol.
Structural formula of Saxagliptin is as follows

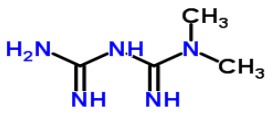


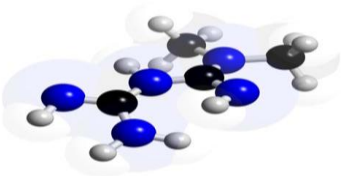


Saxagliptin is used for treatment of type-2 Diabetes Mellitus and also used to improve glycaemic control in type-2 Diabetes Mellitus patients. Saxagliptin is used with Metformin, Sulphonylurea or Pioglytazone, when blood sugar level is not controlled by one of these agents alone. Literature survey reveals that the drug can be estimated only by LC-MS/MS, Spectrophotometric method.

Metformin Hydrochloride

It is water soluble compound and the anti-diabetic drug from the biguanide class of oral hypoglycemic agent. It chemically identify as 1, 1-dimehtylbiguanidine monohydrochloride and empirical formula is $C_4H_{12}N_5CI$, molecular weight is 165.625 gm/mol. Metformin hydrochloride increase glucose transport across the cell membrane in skeletal muscle. Spectroflourimetry, RP-HPLC, HPTLC, LC-MS/MS and UV-Visible Spectroscopy methods was reported for determination of Metformin.
Structural formula of Metformin:





Literature survey reveals that various methods were reported for single estimation of Saxagliptin and Metformin, but no spectroscopic method has been reported for the analysis of

these drugs in combination in tablet dosage form.

Experimental

Reagent and Materials

All the reagents in this assay along with triple distilled water were of analytical grade. Saxagliptin and Metformin were obtained as a gift sample from Dr. Reddy's Lab. Ltd. Riax-M XR 5/500mg tablet were purchased from local market.

Apparatus

Spectral analysis were made on a Jasco Spectrophotometer, Model- V-630 (Japan), was employed with spectral bandwidth of 1nm and wavelength accuracy of ±0.3nm with automatic wavelength correction with a pair of 10mm quartz cells. Glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

Preparation of stock solution

Accurately weighed Saxagliptin and Metformin (10 mg each) was transfer two separate 100ml volumetric flask, dissolved in 50 ml methanol and make up the volume up to the mark. A stoke solution contained 100µg/ml of Saxagliptin and Metformin.

Preparation of working standard

Take required quantity of 100µg/ml stock solution of Saxagliptin and Metformin and diluted with distilled water to obtained working standard of both solution.

Selection of detection wavelength

Solutions of drug were scanned over the range of 200-400nm. It was observed that both the drug showed considerable absorbance at 274nm for Saxagliptin and 231nm for Metformin was selected as the wavelength for detection.

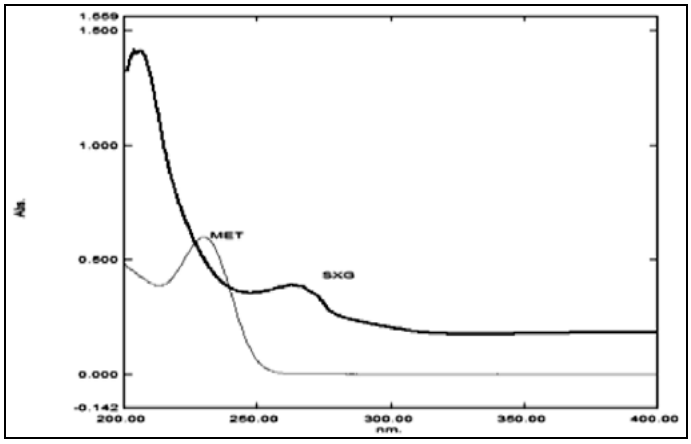


Fig 1: UV Spectra of Metformin and Saxagliptin

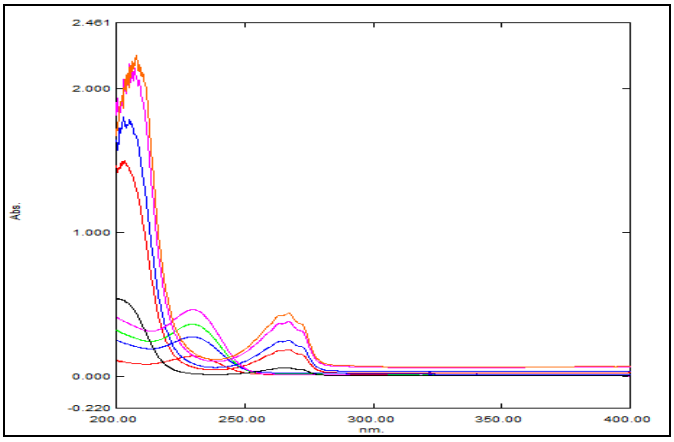


Fig 2: Calibration UV spectra of Metformin and Saxagliptin

Method Validation

Linearity

Working standard solution of Saxagliptin and Metformin was taken in different 10 ml volumetric flasks and diluted up to mark with distilled water to obtained concentrations 50, 60, 70, 80, 90µg/ml of Saxagliptin and 2, 4, 6, 8, 10µg/ml of Metformin. A calibration curve was constructed by plotting concentration versus absorbance and line equation was calculated for both the drugs.

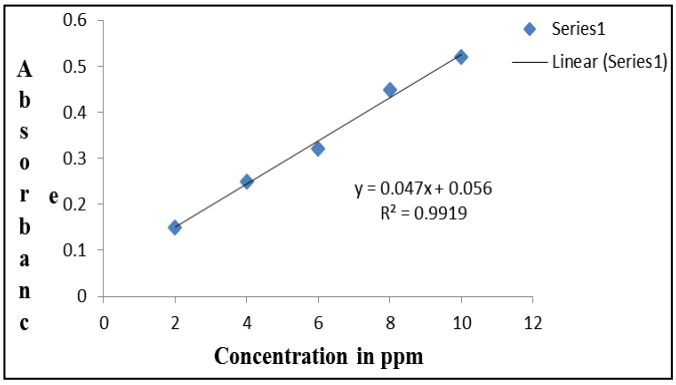


Fig 3: Calibration Curve of Saxagliptin

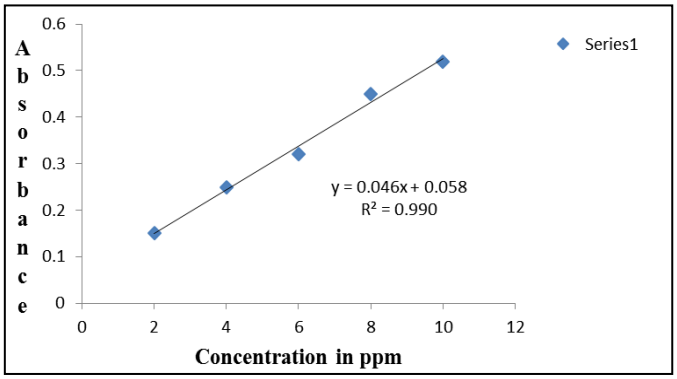


Fig 4: Calibration Curve of Metformin

Precision

The repeatability studies were carried out by estimating response of Saxagliptin (60µg/ml) and Metformin (4µg/ml) five times and results are reported in terms of relative standard deviation. The intermediate precision were carried out by estimating the corresponding responses 3 times on the same day and 3 different days for 3 different concentrations of Saxagliptin (50,60,70 µg/ml) and Metformin (4,6,8 µg/ml) and results are reportred in terms of relative std. deviation.

Accuracy

Recovery studies of Saxagliptin and Metformin were performed to judge the accuracy of the method by standard additions at three different levels 80, 100, 120 %. Mean percentage recovery was determined. Recovery values were calculated shown in table 1.

Table 1: Recovery Studies (API)

Amount of drug sample used Saxagliptin	Obtained(µg) Saxagliptin(n=3)	% Recovery	Amount of drug sample used Metformin	Theoretical amount added (%)	Obtained (µg) metformin(n=3)	% recovery
50µg	40.05	100.12	4µg	80	3.21	100.31
50µg	50.08	100.16	4µg	100	3.99	99.75
50µg	60.3	100.05	4µg	120	4.79	99.90
	Mean % recovery	100.11			Mean % recovery	99.99

Assay of Drug Formulation (Tablet Dosage Form)

Riax-M XR tablet containing 5mg Saxagliptin Hydrochloride and 500mg Metformin Hydrochloride were taken and performed the Weight Variation Test as per I.P. These 20 tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10mg Saxagliptin and Metformin was taken and dissolved in mixture of 50ml methanol and 50ml distilled water in 100ml volumetric flask. Sonicated this solution for 30 minutes and filter the solution. From this solution prepare working solution and the percentage content of the drugs has been found out.

Table 2: Assay of Combined Dosage form (Riax-M tablet)

Drug	Label Claim (mg/Tablet)	Amount Estimated (mg/Tablet)*	Percentage Labelm Claim (%)
Saxagliptin Hydrochloride	5	5	100
Metformin Hydrochloride	500	498	99.60

*****Mean of five reading

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analytic in a sample which can be detected but not necessarily quantitative as an exact value.

$LOD = 3.3\sigma/S$

Where, σ = Relative std. deviation of the response, S = slope of calibration curve.

Quantitation Limit

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determine with suitable precision and accuracy.

$LOQ = 10\sigma/S$

Where, σ = Relative std. deviation of the response, S = slope of calibration curve.

Table 3: Method Validation Parameters

Parameter	Result	
	Metformin	Saxagliptin
Linearity range (µg/ml)	2-10	50-90
Sensitivity(mg/cm2/.001 absorbance unit)	0.016	0.195
Correlation Coefficient (r^2)	0.990	0.987
Slope (m)	0.055	0.012
Intercept (c)	0.031	0.0462
Accuracy	99.99 %	100.11 %
Precision (% RSD)		
Repeatability	0.450	0.687
Intraday	0.31	0.62
Interday	0.88	0.724
LOD (µg)	1.2	7.32
LOQ (µg)	3.6	21.62

Results & Discussion

The developed UV-Visible Spectrophotometric method for the simultaneous estimation of Saxagliptin and Metformin was found to be simple and useful with high accuracy, precision, LOD, LOQ as per ICH guidelines. Sample recoveries in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non interference of formulation excipients in the estimation. In the selected solvent system methanol and distilled water, drugs were stable for more than 48 hours, thus suggesting that samples need not be estimated immediately after collection. The method was successfully used for determination of drugs in their pharmaceutical formulation.

Conclusions

The developed UV-Visible Spectrophotometric method for the simultaneous estimation of Saxagliptin and Metformin in the tablet dosage form in the solvent system methanol and distilled water 1:1 ratio give proper estimation of percentage label claim of marketed product.

Acknowledgement

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