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FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ANAGLIPTIN AND METFORMIN HCl IN BULK AND SYNTHETIC MIXTURE

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ABSTRACT

The present manuscript describes First derivative spectrophotometric method for the simultaneous estimation of Anagliptin and Metformin Hydrochloride in Synthetic Mixture. The first order derivative absorption at 233 nm (zero cross point for Metformin HCl) was used for Anagliptin and 247nm (zero cross point for Anagliptin) was used for Metformin HCl. The linearity was obtained in the concentration range of 5-25 µg/ml for Metformin HCl and 1-5 µg/ml for Anagliptin with correlation coefficient (R2) 0.9999 and 0.9984, respectively. The mean % recoveries were found to be in the range of 99.18-100.44% and 98.18-101.5% for Metformin HCl & Anagliptin, respectively. The suitability of these methods for the quantitative determination of Anagliptin and Metformin HCl was proved by validation. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Anagliptin and Metformin Hydrochloride in their Synthetic Mixture. The results of analysis have been validated statistically and by recovery studies.

Keywords: Metformin HCl, Anagliptin, First derivative spectrophotometric method, Synthetic Mixture.

INTRODUCTION:

Metformin hydrochloride is chemically3-(diaminomethylidene)-1, 1-dimethylguanidine; hydrochloride.[1] Metformin improve hyperglycemia &hyperlipidemia primarily by suppressing glucose production from liver and to lesser extent increases tissue sensitivity to insulin, increase glucose uptake, preventing lipid biosynthesis, promoting fatty acid oxidation. [2] Anagliptin is chemically N-[2-[[2-[(2S)-2 Cyanopyrrolidin-1-yl]-2-oxoethyl]amino]-2-methylpropyl]-2-methylpyrazolo [1,5-a] pyrimidine-6-carboxamide.[3] Anagliptin significantly inhibit the plasma DPP-4 activity and increase the plasma active GLP-1 levels. Anagliptin competitively inhibit dipeptidyl peptidase 4(DPP-4). [4]Metformin Hydrochloride and Anagliptin are available individually in tablet dosage form.But their combination dosage form is not yet available but clinical trial identifier no:25523633 has proven its safety and efficacy in combination. When Metformin and Anagliptin is given

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together the fasting proinsulin: insulin ratio is significantly decreased which in turn improved insulin secretion.[5].Metformin hydrochloride is official in IP[6], BP[7] and USP-NF[8]. Anagliptin is not official in any of the pharmacopoeia. From Literature Survey, various method (Spectroscopic method: UV, Mass Chromatographic method: HPLC, HPTLC) were reported for the analysis of individual drugs and also in combination with other drugs but no method were reported for simultaneous estimation of Metformin HCl and Anagliptin. Hence, the purpose of the present work is to develop and validate first order derivative spectrophotometric method for simultaneous estimation of Metformin HCl and Anagliptin in synthetic mixture.

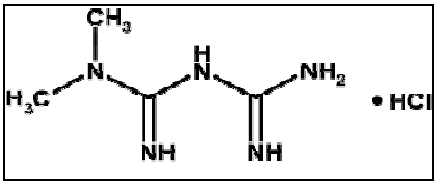

Fig 1: Structure of Metformin HCl

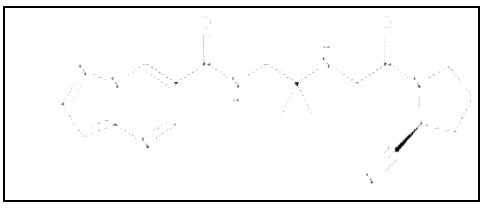

Fig 2: Structure of Anagliptin

MATERIAL AND METHODS:

Instruments:

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents:

The bulk drug, Metformin HCl was received from Wan bury limited Pharmaceuticals, Navi Mumbai and Anagliptin was obtain from Intas Pharmaceuticals, Ahmedabad. Fixed dose of synthetic mixture of Metformin HCl 500 mg and Anagliptin 100 mg were prepared in laboratory scale as pilot batch.

Selection of a Solvent:

Distilled Water was selected as solvent for studying spectral characteristic of drugs.

Preparation of Standard Stock Solution:

Accurately weighed 10 mg of Metformin HCl and 10 mg of Anagliptin each and standard were transferred to two different 100 ml volumetric flask and made up to the mark with Distilled Water to give Final solution containing concentration of 100 µg/ml of Metformin HCl and 100 µg/ml of Anagliptin each. The Volumetric flasks were sonicated for proper solubilization.

Preparation of Working Standard Solution of Metformin HCl and Anagliptin:

From above Stalk solution of Metformin HCl (100 µg/ml) accurately pipette out 0.5, 1.0, 1.5, 2.0, 2.5 ml and transferred it to five 10 ml volumetric flask and made up to the mark with Distilled Water to give Final solution containing concentrations of 5, 10, 15, 20, 25 µg/ml Metformin HCl respectively. From above Stalk solution of Anagliptin(100 µg/ml)accurately pipette out 0.1, 0.2 ,0.3, 0.4, 0.5 ml and transferred it to five 10ml volumetric flask and made up to the mark with Distilled Water to give Final solution containing concentrations 1, 2, 3, 4, 5 µg/ml Anagliptin respectively.

Table 1. Regression analysis data and summary of validation parameters for the proposed method

Parameters	Metformin HCl	Anagliptin
Concentration range (µg/ml)	5-25	1-5
Regression equation	y = -0.0043x - 0.0021	y= 0.0083x - 0.0009
Slope	-0.0043	0.0083
Intercept	0.0021	0.0009
Correlation Coefficient (R^2)	0.999	0.998
Accuracy (% recovery, n=3)	99.18-100.44%	98.18-101.5 %
Repeatability (%RSD, n=6)	1.95	1.68
Intraday (%RSD, n=3)	0.68-1.68	1.45-1.87
Interday (%RSD, n=3)	1.13-1.49	1.16-1.83
LOD (µg/ml)	0.13	0.34
LOQ (µg/ml)	0.41	1.04

Table 2. Recovery data of proposed method

Drug (n=3)	Level (%)	Test amount (µg/ml)	Spiked Amount (µg/ml)	Amount recovered	%Mean recovery ± RSD. (n=3)
Metformin HCl	80	5	4	9.04	100.44 ±1.39
	100	5	5	9.98	99.8 ± 1.29
	120	5	6	10.91	99.18 ± 1.18
Anagliptin	80	1	0.8	1.79	99.44 ± 1.22
	100	1	1	2.03	101.5 ± 1.43
	120	1	1.2	2.16	98.18 ± 1.01

Table 3. Analysis of Metformin HCl & Anagliptin by proposed method

Drug	Amount Taken (mg)	Mean amount found (mg)	% Label claim (n=3)
Metformin HCl	10	10	100
Anagliptin	2	2	100

Selection of Analytical Wavelength:

Standard Concentration range (5-25 µg/ml) solutions of Metformin HCl and Anagliptin (1-5 µg/ml) were prepared in Distilled Water by appropriate dilution and spectrum was recorded between 200-400 nm. All zero order spectrum (D0) were converted to first derivative spectrum (D1) using delta lambda 2.0 and scaling factor 10. The overlain first derivative spectrums of Metformin HCl and Anagliptin at different concentration were recorded. The zero crossing point (ZCP) of Metformin HCl was found to be 233nm and ZCP of Anagliptin was found to be 247nm.

Assay of Synthetic Mixture:

Take 50 mg of Metformin HCl and 10 mg of Anagliptin API in 100 ml volumetric flask and make up to the mark with Distilled Water and sonicated for 10 min. (100 µg/ml)

From this 100 µg/ml solution pipette out 1 ml and transfer it to a 10 ml of volumetric flask and make up to the mark with Distilled Water which will become 10 µg/ml solution of Metformin HCl and 2 µg/ml solution of Anagliptin which consider in the linearity range. Absorbance of a sample solution recorded using first order derivative spectroscopy at 233nm (ZCP of Metformin HCl) and 247nm (ZCP of Anagliptin) for determination of Anagliptin and Metformin HCl, respectively. The analysis procedure was repeated three times with synthetic mixture.

Method Validation:

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

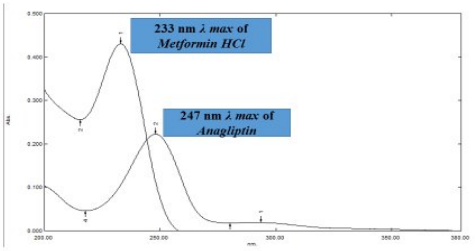

Fig 3: OVERLAIN SPECTRA OF METFORMIN HCl (5 µg/ml) AND ANAGLIPTIN (1 µg/ml) IN DISTILLED WATER (ZERO ORDER)

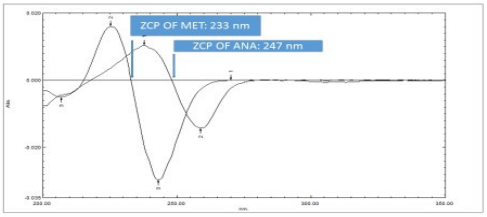

Fig 4: OVERLAIN SPECTRA OF METFORMIN HCl (5 µg/ml) AND ANAGLIPTIN (1 µg/ml) IN DISTILLED WATER (FIRST ORDER)

Linearity (Calibration curve):

Appropriate volume of aliquot from Metformin HCl and Anagliptin standard stock solution was transferred to 10 ml volumetric flask. The volume was made up to the mark with Distilled Water to give solution containing 5-25 µg/ml Metformin HCl and 1-5 µg/ml Anagliptin. All D1spectrums were recorded using above spectrophotometric condition. D1 absorbance at 233 nm and 247 nm were recorded for Anagliptin and Metformin HCl, respectively (n=5). Calibration curve were

constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Anagliptin y=0.0083x-0.0009 (R^2 = 0.9984) and Metformin HCl was y = -0.0043x - 0.0021

R^2 = 0.9999.

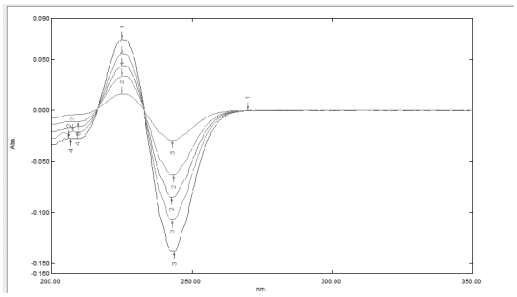

Fig 5: OVERLAIN SPECTRA OF METFORMIN HCl (5-25 µg/ml)

Accuracy:

Accuracy was assessed by determination of the recovery of the method by addition ofstandard drug to the prequantified sample preparation at three different concentration levels 80 %, 100 % and 120 %, taking in to consideration percentage purity of added drug sample. The amounts of Metformin HCl and Anagliptin were estimated by applying obtained values to the respective regression line equations. Each concentration was analyzed 3 times and average recovery were measured.

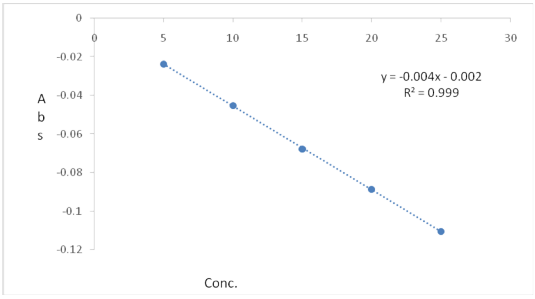

FIG 6: CALIBRATION CURVE OF METFORMIN HCl

Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

The repeatability was evaluated by assaying 6 times of sample solution of 15 µg/ml Metformin HCl and 3 µg/ml Anagliptin prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Metformin HCl & Anagliptin was carried out by estimating different concentration of Metformin HCl (10, 15, 20 µg/ml) and Anagliptin (2, 3, 4 µg/ml), 3 times on same day and on 3 different day (first, second and third).

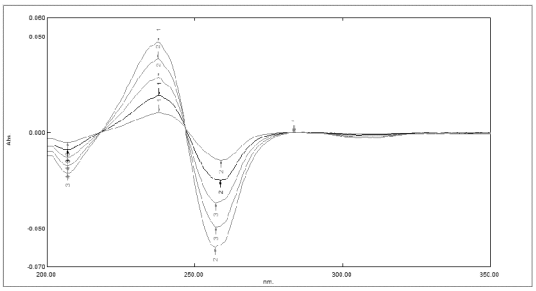

Fig 7: OVERLAIN SPECTRA OF ANAGLIPTIN (1-5 µg/ml)

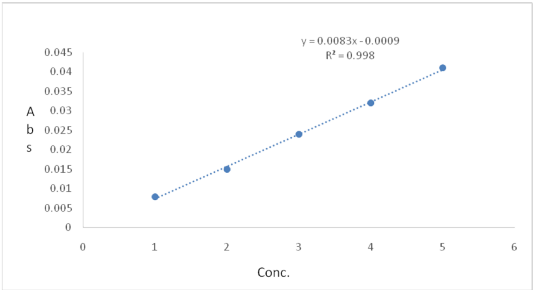

FIG 8: CALIBRATION CURVE OF ANAGLIPTIN

Limit of Detection (LOD) and Limit of Quantification (LOQ):

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

RESULT AND DISCUSSION:

A reliable first order derivative spectrophotometric method was developed for simultaneous estimation of Metformin HCl &

Anagliptin in synthetic mixture by UV Spectrophotometry. Beers law was obeyed in concentration range of 5-25 µg/ml for Metformin HCl and 1-5 µg/ml for Anagliptin at 247 nm and 233 nm wavelengths, respectively. The correlation coefficient Metformin HCl & Anagliptin was found to be R2= 0.9999and 0.9984. The mean % recoveries were found to be in the range of % 99.18-100.44% and 98.18-101.5%, respectively. Precision (% RSD) of Metformin HCl & Anagliptin was found to be 1.13- 1.68 % & 1.16-1.87 % respectively. The LOD and LOQ were 0.137 µg/ml and 0.41 µg/ml Metformin HCl and 0.345 µg/ml and 1.04 µg/ml of Anagliptin, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analytes, which can be applied for the analysis of Metformin HCl & Anagliptin in synthetic mixture.

CONCLUSION:

The results of present study indicate that the proposed UV spectrophotometric method is simple, rapid, precise and accurate. The developed UV spectrophotometric method was found suitable for determination of Metformin HCl & Anagliptin in bulk drug and syntheticmixture without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of Metformin HCl & Anagliptin in combination. It can therefore be concluded that the developed analytical method was precise & accurate and can be used for routine Analysis of both the drug in combination.

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REFERENCES:

1. Medicinenet, "Metformin: Drug Profile" http://www.medicinenet.com/Metformin/index.html
2. Mohan H. Textbook of Pathophysiology; 6th Edn; Jaypee Brothers, Medical publishers Pvt Limited, Chandigarh, pp 819.
3. Chemspider, "Anagliptin chemical-structure" www.chemspider.com/chemical structure, html
4. Rang H., and Dale M. Rang & Dale's pharmacology; 7th Edition; Elsevier Publication, Toronto, 2012, pp 377-378.
5. Takeuchi Y, "Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of E3024, a Novel and Selective Dipeptidyl Peptidase-IV Inhibitor, in Healthy Japanese Male Subjects: Rash Development in Men and Its Possible Mechanism." Sci Pharm. 2013, 4, 663-678.
6. Indian Pharmacopoeia - 2014, Government of India Ministry of health & family welfare, Published by Indian Pharmacopoeia Commission, 2014, Vol. 2, pp 503, 728.
7. British Pharmacopoeia- 2010, The Stationary Office on Behalf of The Medicine & Healthcare Products Regulatory Agency(MHRA), London, United Kingdom, 2009, Vol.2, pp-3813.
8. United States Pharmacopoeia 30, National Formulary 25, United States Pharmacopoeia Convention, Rockville, 2010, pp 1092.

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