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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ANAGLIPTIN AND METFORMIN HCl BY SECOND DERIVATIVE METHOD

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ABSTRACT: A Simple, accurate, and precise spectrophotometric method (1st order derivative method) was developed and validated for the estimation of Anagliptin and Metformin HCl in synthetic mixture. In this method, Anagliptin was estimated at 244 nm where Metformin HCl showed zero absorbance. Metformin HCl is estimated at 234 nm at which Anagliptin showed zero absorbance. The method was linear over the concentration ranges 2-12 µg/mL for Anagliptin and 5-30 µg/mL for Metformin HCl. The LOD was found to be 0.127 µg/mL for Anagliptin and 0.599 µg/mL for Metformin HCl. The LOQ was found to be 0.387 µg/mL for Anagliptin and 1.817µg/mL for Metformin HCl. In the recovery study, % recovery was 99.55 – 101.65% of Anagliptin and 100.25-100.57% of Metformin HCl. The method was found to be precise as %RSD was less than 2.00 in repeatability, Interday, and intraday precision for Anagliptin and Metformin HCl. The % assay of analyte drugs was found to be 101.42% of Anagliptin and 99.61% of Metformin HCl, which showed good applicability of the developed method.

INTRODUCTION: Anagliptin is used for type 2 diabetes mellitus and longer duration of action for treatment of type 2 non-insulin dependent diabetes mellitus disease as a Dipeptidyl Peptidase 4 inhibitor [1-6]. Anagliptin N-[2-[[2-[(2S)-2-Cyano-pyrrolodin-1-yl]-2-oxoethyl] amino]-2-methylpro-pyl]- 2- methylpyrazolo [1, 5-a] pyrimidine- 6-carboxamide **Fig. 1** is a Dipeptidyl Peptidase 4 inhibitor which is used in treatment of type 2 NIDDM [7].

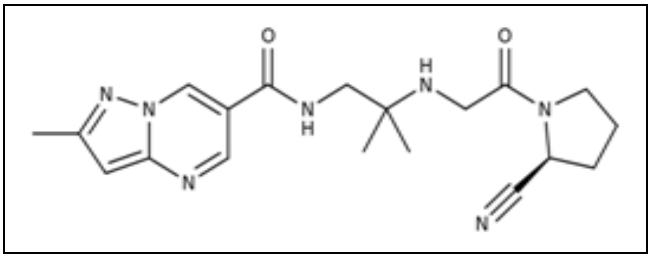

FIG. 1: CHEMICAL STRUCTURE OF ANAGLIPTIN

Dipeptidyl Peptidase 4 enzyme breaks down the incretins GLP-1 gastrointestinal hormones released in response to a meal. By preventing GLP-1 inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards the normal level. This drug is not official in any of the pharmacopeias. A literature survey revealed that few methods are

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reported for determination of ANA, either alone or in combination [8], by spectrophotometric [9-11], HPLC [12], LC/MS [13].

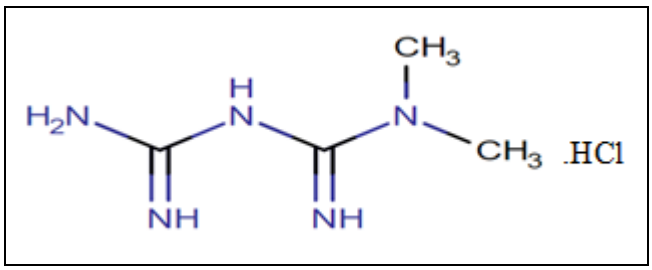

FIG. 2: CHEMICAL STRUCTURE OF METFORMIN HCl

Metformin is chemically a1-Caramimidamido-N, N-Dimethylmethanimidamide **Fig. 2,** and has pharmacologically is based on the Biguanides category. It suppresses hepatic gluconeogenesis and glucose output from the liver. This is the major action responsible for lowering blood glucose in diabetics. It is official in IP [14], BP [15], and USP [16]. Literature review reveals that many spectro-photometric [17], HPLC [18], HPTLC [19] methods are reported for determination of MET, either alone or in combination [8].

The aim of the present work was to develop a spectrophotometric method of simultaneous estimation of ANA and MET in combination. It is pertinent to note that some of the published methods enabled estimation of drugs in combination products containing two drugs via zero and first-order derivative spectrophotometric method and HPLC; however, so far, not any second-order derivative spectrophotometric method was reported for the same. Hence, to achieve this aim, an accurate second derivative method has been developed and successfully applied to the pharmaceutical dosage form.

MATERIALS AND METHODS:

Apparatus: The instrument used was of Shimadzu UV-1600 series with a pair of 1 cm matched quartz cells. The software used was UV Probe 4.2 series. A digital analytical balance (Wenstar DA14-222) and ultrasonic sonicator (Equitron) were used in the study. Pipettes of 1, 2,5,10 mL; volumetric flasks of 10,100 mL; beakers of 100, 250, 500 mL were made up of Borosil glass.

Chemicals and Reagents: Drug samples of ANA and MET were provided as a gift sample by Intas Pharmaceutical Pvt. Ltd., Ahmedabad, India. Solvents like Distilled water were from E. Merck, Mumbai. All the chemical reagents were of analytical grade.

Preparation of Standard Stock Solution: Accurately weighed quantity of 10 mg of ANA and 10 mg of MET were transferred into 100 mL volumetric flask individually. Initially, about 50 mL distilled water was added to the flask, respectively, and sonicated. The volume was made up to the mark with distilled water to prepare stock solutions to correspond to 100 µg/mL of ANA and 100 µg/mL of MET.

Determination of Wavelength for Second Order Derivative Method: The second-order derivative method was selected because the spectral characteristics and resolution were good in the second derivative spectra. The zero-crossing points of ANA 234 nm and for MET 244 nm were selected based on linearity data. At 234 nm ANA showed zero absorbance, but MET had considerable absorbance. Similarly, at 244 nm MET showed zero absorbance, but ANA had a considerable amount of absorbance.

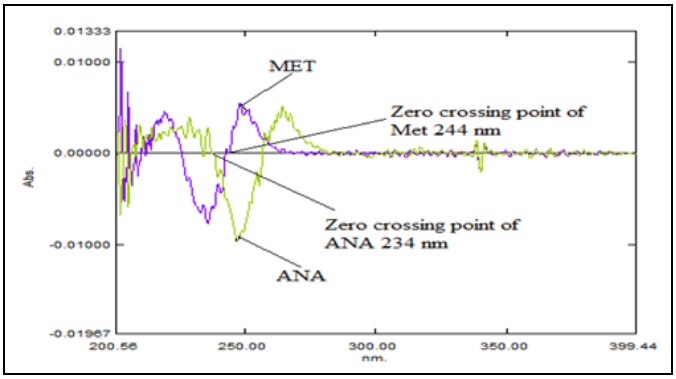

FIG. 3: OVERLAIN SPECTRA OF ANA AND MET IN INDIVIDUAL SOLUTION (10 µg/mL) SHOWING THEIR ZERO CROSSING POINTS

Preparation of Test Solution for Assay Determination: Twenty tablets were accurately weighed, their average weight was calculated. The amount of finely powdered tablet equivalent to 2 mg ANA and 10 mg MET were weighed and transferred into a 100 mL volumetric flask, and the volume was adjusted to mark with distilled water. The contents of the flask were sonicated for 15 min to dissolve the active ingredients completely. The solution was filtered through a Whatman filter paper no. 41. From this 1 mL aliquot was transferred into a 10 mL volumetric flask, and the

volume was made up with distilled water. This test solution containing working concentrations of 2 µg/mL ANA and 10 µg/mL MET, respectively, in the mixture was analyzed for assay determination.

Preparation of Calibration Curve: From working standard solution of ANA (100 µg/mL), aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL were transferred into series of 10 mL volumetric flask and diluted up to mark with distilled water. This yielded solutions of 2, 4, 6, 8, 10, and 12 µg/mL of ANA. From the standard working solution of MET (100 µg/mL), aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL were transferred into series of 10 mL volumetric flask and diluted up to mark with distilled water. This yielded solutions of 5, 10, 15, 20, 25, and 30 µg/mL of MET.

Method Validation: The proposed method was validated as per ICH guidelines Q2 R1 [20].

Linearity and Range: Linearity was studied by preparing standard solutions at 6 different concentrations. The linearity range for ANA and MET was found to be 2-12 µg/mL and 5-30 µg/mL, respectively. For each solution, the absorbance of ANA was measured at 244 nm i.e., ZCP of MET, and vice versa the absorbance of MET was measured at 234 nm *i.e.,* ZCP of ANA. The calibration curves of absorbance versus concentration were plotted. The linearity of absorbance responses versus concentrations was demonstrated by linear regression analysis.

Precision: The precision of the proposed method was assessed as repeatability, intra-day precision, and inter-day precision. Repeatability was performed by applying six replicates of sample analysis. For intermediate precision, Intraday and Interday precision was performed by determining corresponding responses of six replicates on the same and different days for ANA (2 µg/mL) and for MET (10 µg/mL). The results were reported in terms of %RSD.

Accuracy: Recovery studies were carried out by standard addition method. A known amount of standard ANA (1, 2 and 3 µg/mL) and MET (5, 10, and 15 µg/mL) similar to 50%, 100%, and 150% of the label claim were added to test solution of ANA (2 µg/mL) and MET (10 µg/mL).

The same study was carried out three times at each level of recovery.

LOD and LOQ: The LOD and LOQ of the developed method were calculated from the calibration curve using an equation, LOD = 3.3* σ/S, and LOQ = 10* σ/S. Where σ = the standard deviation of y-intercepts of regression lines of six calibration curves, S = the average of the slopes of six calibration curves.

RESULTS AND DISCUSSION:

Linearity: Aliquots of standard solution were applied in the concentration range 2-12 µg/mL and 5-30 µg/mL for ANA and MET, respectively. The calibration curve obtained by the least square regression analysis between average absorbance and concentration showed a linear relationship with a correlation coefficient R^2nearer to 0.99 for ANA at 244 nm, and for MET at 234 nm, respectively. The linear regression equation obtained were y = -0.000596x - 0.000031 and y = -0.000861x + 0.002552 for ANA and MET respectively **Fig. 4, Fig. 5, Fig. 6, Fig.7, Table 1**.

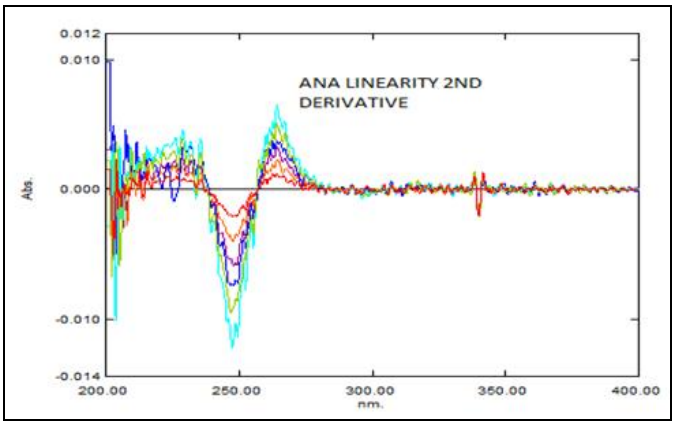

FIG. 4: OVERLAIN SPECTRA FOR LINEARITY OF ANA (2–12 µg/mL) AT ZCP OF MET 244 nm

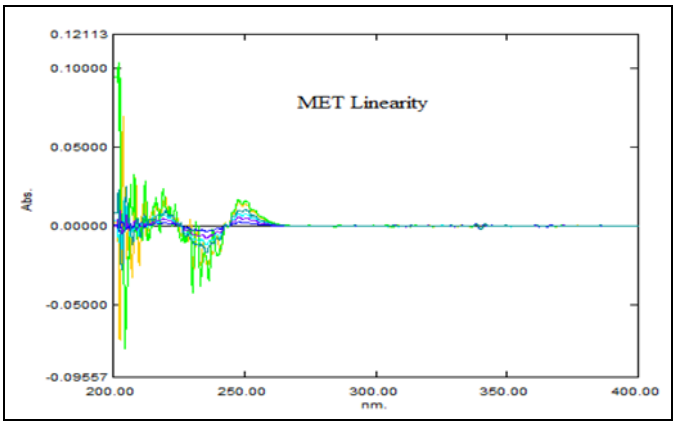

FIG. 5: OVERLAIN SPECTRA FOR LINEARITY OF MET (5-30 µg/mL) AT ZCP OF ANA 234 nm

TABLE 1: LINEARITY DATA OF ANA AND MET

S. no.	ANA			MET		
	Conc. (µg/mL)	Absorbance* ± SD	%RSD	Conc. (µg/mL)	Absorbance* ± SD	%RSD
1	2	-0.00131 ± 0.000023	1.76	5	-0.00247 ± 0.000096	0.93
2	4	-0.00248 ± 0.000028	0.93	10	-0.00573 ± 0.000082	1.27
3	6	-0.00348 ± 0.000034	0.66	15	-0.00985 ± 0.000089	0.98
4	8	-0.00465 ± 0.000051	0.49	20	-0.01446 ± 0.000076	1.10
5	10	-0.00594 ± 0.000063	0.38	25	-0.01863 ± 0.000090	1.05
6	12	-0.00733 ± 0.000079	0.31	30	-0.02394 ± 0.000081	1.039

* Average of six determinations

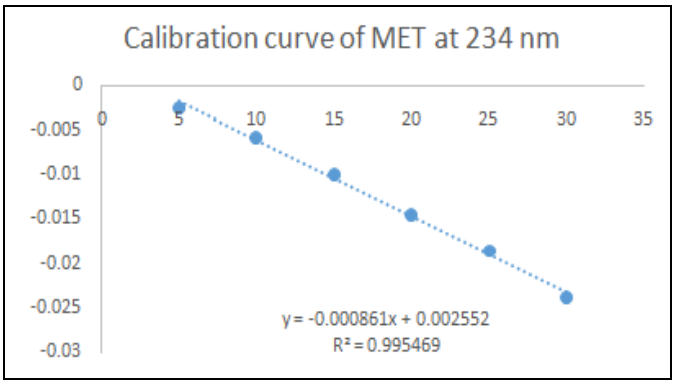

FIG. 6: CALIBRATION CURVE OF MET AT 234 nm

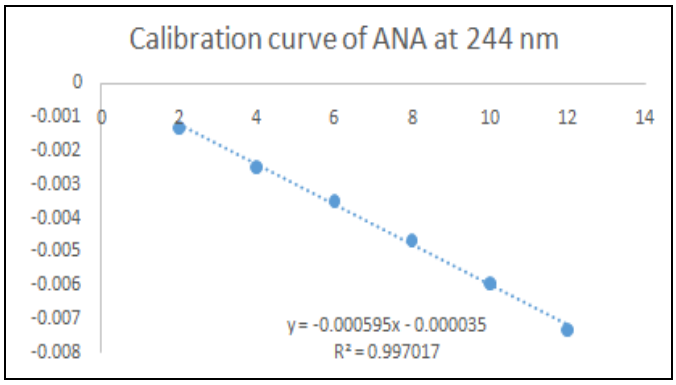

FIG. 7: CALIBRATION CURVE OF ANA AT 244 nm

Precision: The %RSD of repeatability was found to be 1.60 for ANA and 1.08 for MET. The %RSD of Intraday precision was found to be 1.43 for ANA and 1.16 for MET. The % RSD of Interday precision was found to be 1.65 for ANA and 1.40 for MET. Thus, confirming the precision of the method **Table 2**.

TABLE 2: REPEATABILITY, INTRADAY AND INTERDAY PRECISION OF ANA AND MET

	Concentration (µg/mL)		Absorbance* ± SD		%RSD	
	ANA	MET	At 244 nm	At 234 nm	At 244 nm	At 234 nm
Repeatability	2	10	-0.00131 ± 0.00002	-0.00602 ± 0.00007	1.60	1.08
Intraday Precision	2	10	-0.00131 ± 0.00003	-0.00597 ± 0.00006	1.43	1.16
Interday Precision	2	10	-0.00130 ± 0.00002	-0.00599 ± 0.00008	1.65	1.40

*Average of six determinations

Accuracy: Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for ANA was in the range of 99.5526 - 101.65%, while for MET, it was found to be in the range of 100.286 - 100.572% **Table 3**.

TABLE 3: ACCURACY DATA OF ANA AND MET

Drug	Amount of Test Solution (µg/mL)	Amount of Std added (µg/mL)	Absorbance* ± SD	Total Amount Found (µg/mL)	Recovered amount (µg/mL)	% Recovery	% RSD
ANA	2	0	-0.0008866 ± 0.000015	2.0078	--	100.391	1.276
	2	1	-0.0014833 ± 0.000011	3.0089	1.0089	100.298	0.643
	2	2	-0.0021133 ± 0.000020	4.0659	2.0659	101.65	0.859
	2	3	-0.0026566 ± 0.000035	4.977	2.977	99.552	1.183
MET	10	0	-0.0061333 ± 0.000057	10.087	--	100.874	0.664
	10	5	-0.0104 ± 0.000100	15.042	5.042	100.286	0.772
	10	10	-0.014766 ± 0.000152	20.114	10.114	100.572	0.882
	10	15	-0.019066 ± 0.000208	25.108	15.108	100.435	0.962

*Average of six determinations

LOD & LOQ: The LOD, as calculated by standard formula as given in ICH guidelines, was found to be 0.127 µg/mL and 0.599 µg/mL for ANA and MET, respectively. The LOQ, as calculated by standard formulae as given in ICH guidelines, was found to be for 0.387 µg/mL and 1.817 µg/mL ANA and MET, respectively.

Analysis of ANA and MET in Tablet Formulation: The developed methods were applied to tablet preparation. The % Assay of ANA and MET was 101.42 ± 1.169% and 99.61 ± 0.427%, respectively, of the labeled amount **Fig. 8, Table 4**.

TABLE 4: ANALYSIS OF TABLET FORMULATION

Drug	Amount of drug actual	Amount of drug estimated	% Label claimed* ± SD	% RSD
ANA	2	2.0285	101.42 ± 1.169	1.152
MET	10	9.9611	99.61 ± 1.273	1.277

*Average of six determinations

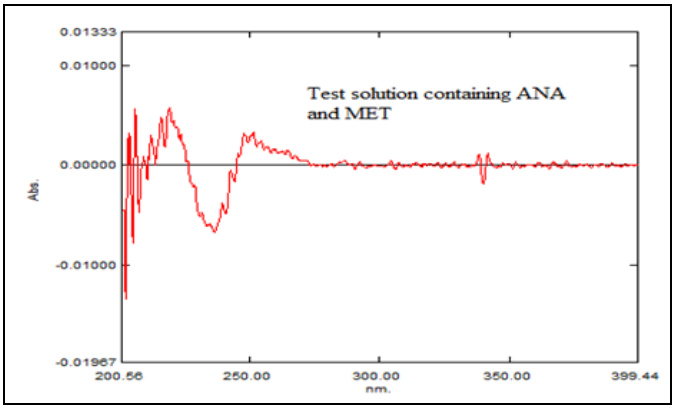

FIG. 8: SPECTRA OF TEST SOLUTION OF ANA (2 µg/mL) AND MET (10 µg/mL)

TABLE 5: SUMMARY OF VALIDATION PARAMETERS

S. no.	Parameter	ANA (244 nm)	MET (234 nm)
1	Specificity	Specific	Specific
2	Linearity Range	2-12 µg/mL	5-30 µg/mL
3	Regression Line equation	y = -0.000596x - 0.000031	y = -0.000861x + 0.002552
4	Correlation Coefficient	R^2 = 0.997	R^2 = 0.995
5	Precision		
	Repeatability	1.60	1.08
	Intraday Precision	1.43	1.16
	Interday Precision	1.65	1.40
6	Accuracy	99.55 – 101.65 %	100.25-100.57 %
7	LOD (µg/mL)	0.127 µg/mL	0.599 µg/mL
8	LOQ (µg/mL)	0.387 µg/mL	1.817µg/mL

CONCLUSION: The proposed spectrophotometric method is precise, specific, linear, and accurate for the estimation of ANA and MET in the pharmaceutical dosage form without interference from the excipients. The developed method is validated as per ICH guidelines. The method was successfully used for the simultaneous estimation of both drugs in the presence of each other.

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CONFLICTS OF INTEREST: Nil

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