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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND TELMISARTAN IN COMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

Four simple, rapid, precise, economical and accurate spectrophotometric methods have been developed for simultaneous analysis of Metoprolol succinate and Telmisartan in their combined dosage form. Method 1, First derivative simultaneous equation method (Vierodt's method). It employs formation and solving of simultaneous equation using two wavelengths 230.2 nm (λmax of Metoprolol succinate) and 237 nm (λmax of Telmisartan) in first derivative spectra. Method 2, First derivative Q-Absorbance equation method. It involves, formation of Q-absorbance equation at 231.8 nm (isoabsorptive point) and 237 nm (λmax of Telmisartan) in first derivative spectra. Method 3, Absorbance correction method, involves measurement of absorbance at 296.6 nm for estimation of TEL and measurement of corrected absorbance at 223 nm for estimation of MET. Method 4, Combination of First derivative dual wavelength ,which uses the difference in absorbance at 282.4 nm and 284.6 nm for estimation of MET and zero crossing first derivative spectrophotometry involves measurement of amplitudes at 330 nm for estimation of TEL in first derivative spectra. Developed methods were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 3-20 µg/ml for MET and 4-16 µg/ml for TEL with R square value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 99 to 101%. Intraday and interday precision was checked for all methods and mean %RSD was found to be less than 2 for all the methods. The methods were successfully applied for estimation of MET and TEL in marketed formulation.

INTRODUCTION: Metoprolol succinate [MET] chemically is 1- (isopropyl amino)- 3- [4- (2-methoxyethyl) penoxy] propan-2-ol **(Fig. 1)**. It is used as b1 blocker. This preferential effect is not absolute, however, and at higher plasma concentrations, MET also inhibits b_2-adrenorecptors, chiefly located in the bronchial and vascular musculature. The drug is official in USP and BP [1-2]. Telmisartan chemically is 2-(4-{[4-Methyl-6-(1-methyl-1H-1, 3-benzodiazol-2-yl)-2-propyl -1H-1, 3-benzodiazol-1-yl]methyl}phenyl)benzoic acid **(Fig. 2)**. The drug is official in BP [1]. It is angiotensin II receptor antagonist, effective in the treatment of hypertension. It is also effective when used alone or in combination with other drugs for the treatment of high blood pressure.

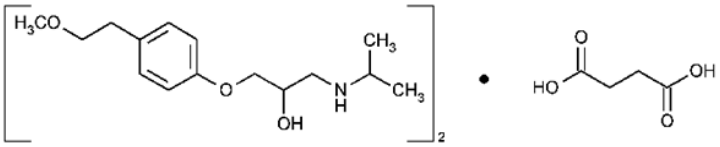


FIG. 1: METOPROLOL SUCCINATE

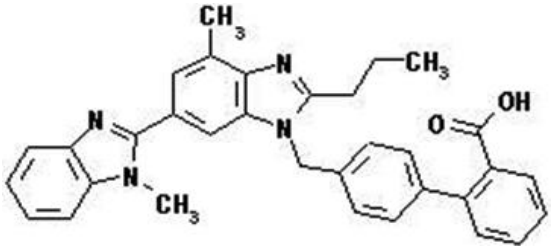


FIG. 2: TELMISARTAN

Objective of study: Survey of literature revealed that numbers of method have been reported in literature for the individual analysis of Metoprolol succinate and Telmisartan by UV spectrophotometric method [3-5] like area under curve, second order derivative spectroscopy and RP-HPLC method [6-7].RP-HPLC method available in literature for simultaneous determination of Metoprolol with Amlodipine [8] and Hydrochlorthiazide [12]. RP-HPLC method available in literature for simultaneous determination of Telmisartan with Amlodipine [9], Indapamide [10] and Atrovastatin [11]. However, to our knowledge, there is no reported uv-spectrophotometric method available for simultaneous estimation of Metoprolol succinate and Telmisartan.

So, the aim of the present work was to develop easy, economic, accurate, specific and precise spectrophotometric methods for simultaneous estimation of Metoprolol succinate and Telmisartan in bulk drugs and combined pharmaceutical formulations and validation of newly developed analytical methods.

MATERIALS AND METHODS:

Apparatus and Software: Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UVProbe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm.

Reagents and Chemicals:

Solvent: Methanol analytical reagent grade (Spectrochem Pvt. Ltd, Mumbai, India).

Diluent: Methanol analytical reagent grade (Spectrochem Pvt. Ltd, Mumbai, India).

Year of Experiment– 2011

Site- Quality Assurance Laboratory, Centre of Relevance and Excellence in Novel Drug Delivery System, G. H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara – 390 002, Gujarat, India.

Preparation of Stock Solution: Accurately weighed MET and TEL (in quantities of 12.5 mg and 10 mg respectively) were transferred to two separate 25 ml volumetric flasks, dissolved with the use of methanol and volume was made up to the mark with methanol to obtain stock solution of MET (500 µg/ml) and TEL (400 µg/ml)

Preparation of Working Standard Solution: From this, standard stocks solutions of MET (50 µg/ml) and TEL (40 µg/ml) were prepared by transferring 2.5 ml aliquots to other 25 ml volumetric flasks and making up the volume with methanol.

Preparation of Calibration Curve of Standard MET and TEL: From working std. solution of MET (50 µg/ml) 0.75, 1, 1.25, 1.5, 1.75 and 2 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 3.75 to 10 µg/ml of MET. From working std. solution of TEL (40 µg/ml) 1.5, 2, 2.5, 3, 3.5 and 4 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 6 to 16 µg/ml of TEL.

Method 1-

First derivative simultaneous equation method (Vierodt's method): If a sample containing two absorbing drug (X and Y) each of which absorbs at λmax of other. It may possible to determine both drugs by the technique of simultaneous equations (Vierodt's method) provided that certain criteria apply. The information required is the aborptivities of X at and λ1 and λ2 ax1 and ax2 respectively (a) The aborptivities of Y at and λ1 and λ2 ay1 and ay2 respectively (b) The absorbances of the diluted sample at λ1 and λ2 , A1 and A2 respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon

the fact that at λ1 and λ2 the absorbance of the mixture is the sum of the individual absorbance of X and Y. From the stock solutions, standard solutions of MET (7.5 µg/ml) and TEL (12 µg/ml) were prepared by appropriate dilution and were scanned in the entire UV range 220 to 400 nm and were stored in the memory of the instrument and transformed to first derivative with Δλ = 4 nm and scaling factor 50 **(Fig. 3).**

Wavelengths with maximum absorbance (λmax) for MET and TEL are 230.2 nm and at 237 nm, respectively in first derivative spectra. The wavelengths selected for analysis were 230.2 nm and 237 nm respectively. A series of standard solutions ranging from 3.75-10 µg/ml for MET and 6-16 µg/ml for TEL were prepared and the absorbance of solutions was recorded at 230.2 and 237 nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in the concentration range under study **(Fig. 4)**.

The concentration of two drugs in mixture was calculated by using following equations:

$C\,x = (A_1\,aY_2 - A_2\,Ay_1) / (aX_1\,aY_2 - aX_2\,aY_1)$..... (1)

$C\,y = (aX_1\,A_2 - aX_2\,A_1) / (aX_1\,aY_2 - aX_2\,ay_1)$..... (2)

Where; Cx & Cy are concentrations of MET and TEL respectively in gm/100 ml in the sample solution.

A_1 & A_2 are the absorbances of the mixture at 230.2 nm & 237 nm respectively; aX_1 and aX_2 = Absorptivity of MET at 230.2 nm and 237 nm; aY_1 and aY_2 = Absorptivity of TEL at 230.2 nm and 237 nm

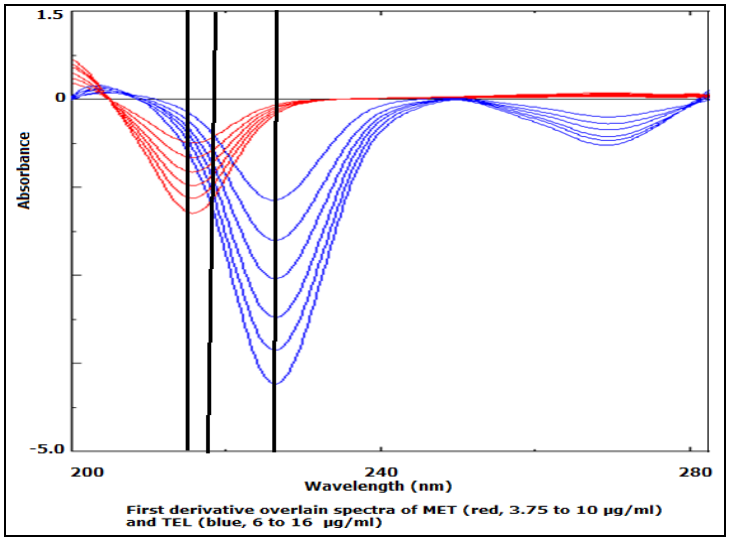


FIG. 3: FIRST DERIVATIVE OVERLAIN SPECTRA OF MET (3.75, 5, 6.25, 7.5, 8.75, 10 µg/ml, red) and TEL (6, 8, 10, 12, 14, 16 µg/ml, blue)

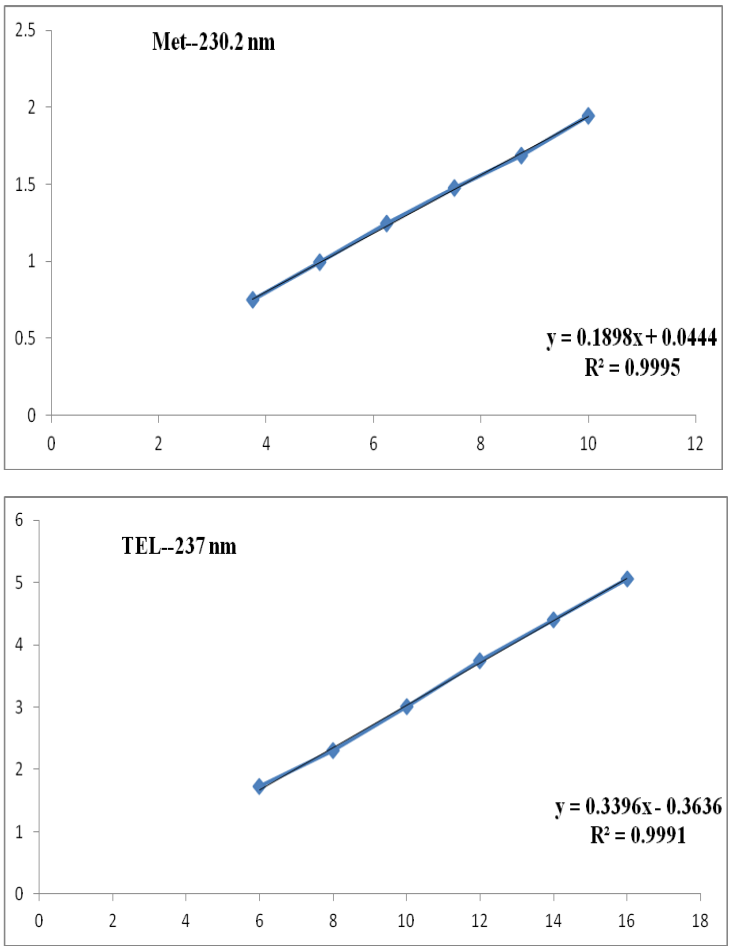


FIG. 4. CALIBRATION GRAPHS OF MET AND TEL BY FIRST DERIVATIVE SIMULTANEOUS METHOD

Method 2-

First derivative Q-Absorbance ratio method: Q method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two compounds. From the stock solutions, standard solutions of MET (7.5 µg/ml) and TEL (12 µg/ml) were prepared by appropriate dilution and were scanned in the entire UV range 220 to 400 nm and were stored in the memory of the instrument and transformed to first derivative with Δλ = 4 nm and scaling factor 50 **(Fig. 5).**

The maximum absorbance (λmax) and isoabsorptive point were determined. MET and TEL have λmax at 230.2 nm and at 237 nm, respectively in first derivative spectra. Both the drugs were found to have same absorbance at 231.8 nm (isoabsorptive point). The wavelengths selected for analysis were 231.8 nm and 237 nm respectively.

A series of standard solutions ranging from 3.75-10 µg/ml for MET and 6-16 µg/ml for TEL were prepared and the absorbance of solutions was recorded at 231.8 and 237 nm to plot a calibration curve of absorbance versus concentration **(Fig. 6)**. Calibration curves were found to be linear in the concentration range under study. Absorptivity values of MET and TEL were determined at selected wavelengths and are presented in Table. The concentration of two drugs in mixture was calculated by using following equations:

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \quad \text{..... (3)}$$

$$C_Y = (A_1/aX_1) - CX \quad \text{..... (4)}$$

Where; Qm = A_2/A_1, Qx = ax_2/ax_1, Qy = ay_2/ay_1; **1** designates isoabsorptive point and **2** designates λ-max of TEL; ax_1 and ax_2 is Absorptivity of MET at 1 and 2 wavelength respectively; $ay_1$and ay_2 is Absorptivity of TEL at 1 and 2 wavelength respectively; A_1 and A_2 are absorbances of the mixture at 1 and 2 wavelength respectively.

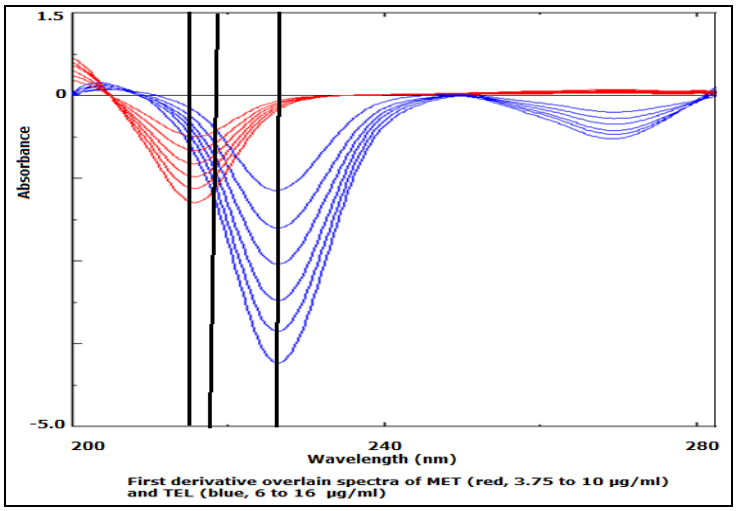
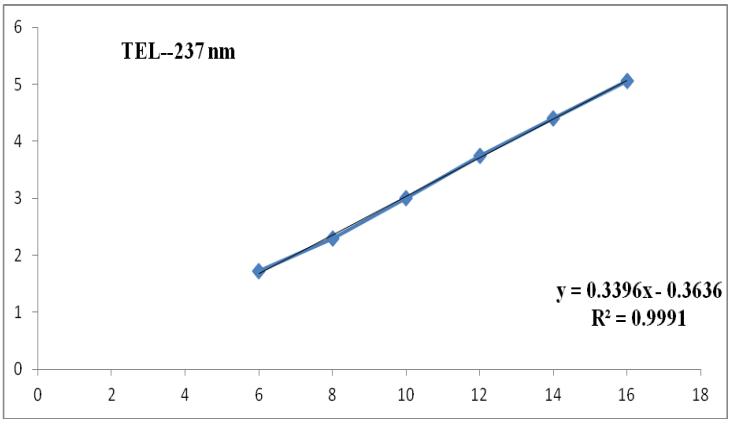
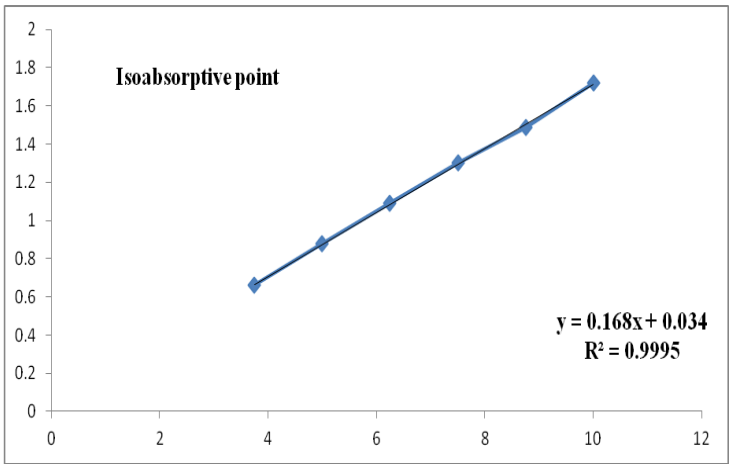


FIG. 5. FIRST DERIVATIVE OVERLAIN SPECTRA OF MET (3.75, 5, 6.25, 7.5, 8.75, 10 µg/ml, red) and TEL (6, 8, 10, 12, 14, 16 µg/ml, blue)





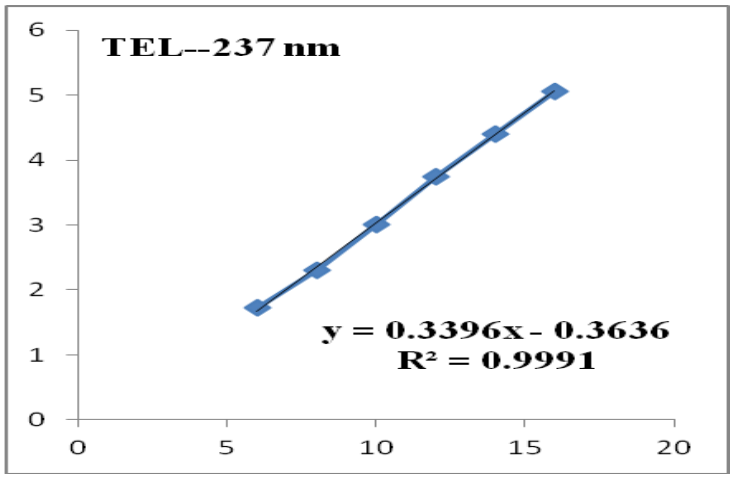


FIG. 6: CALIBRATION GRAPHS OF MET AND TEL BY FIRST DERIVATIVE ABSORBANCE Q-EQUATION METHOD

Method 3-

Absorbance correction method: Absorbance spectra of MET (3.75-10 µg/ml) and TEL (6-16 µg/ml) in the range of 220 to 400 nm were taken. Overlain zero order spectra of both drugs are shown below **(Fig.7)**. This method involves measurement of absorbance at 296.6 nm and 223 nm. At 296.6 nm, MET shows no absorbance and TEL can be estimated directly by calibration curve without any interference of MET. MET shows maximum absorbance at 223 nm where TEL is having considerable interference. So, absorbance of TEL at 241.2 nm is corrected from total absorbance and then it is related to concentration of MET. Calibration graphs are prepared at 296.6 nm and 223 nm for TEL and MET respectively shown below (**Fig. 8)**.

$$CA_{MET,\ 223\ nm} = A_{223\ nm} - A_{TEL,\ 223\ nm}$$

$CA_{MET,\ 223\ nm}$ = Corrected absorbance for MET at 223 nm; $A_{223\ nm}$ = Absorbance at 223 nm; $A_{TEL,\ 223\ nm}$ = Absorbance of TEL at 223 nm

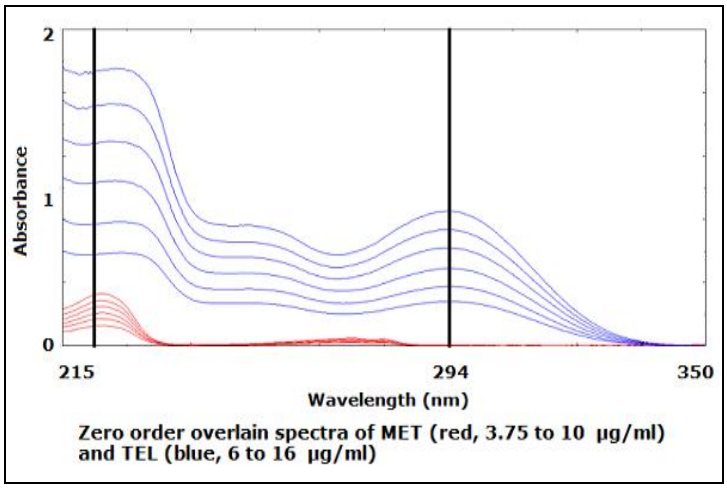
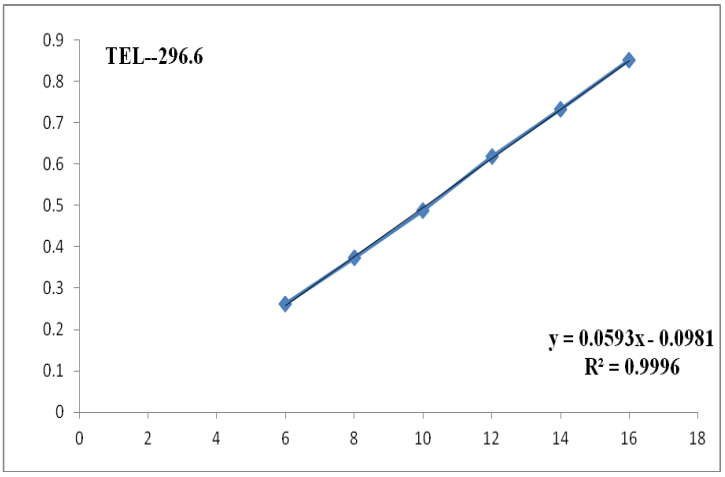


FIG. 7: ZERO ORDER SPECTRA OF MET (3.75, 5, 6.25, 7.5, 8.75, 10 µg/ml, red) and TEL (6, 8, 10, 12, 14, 16 µg/ml, blue)



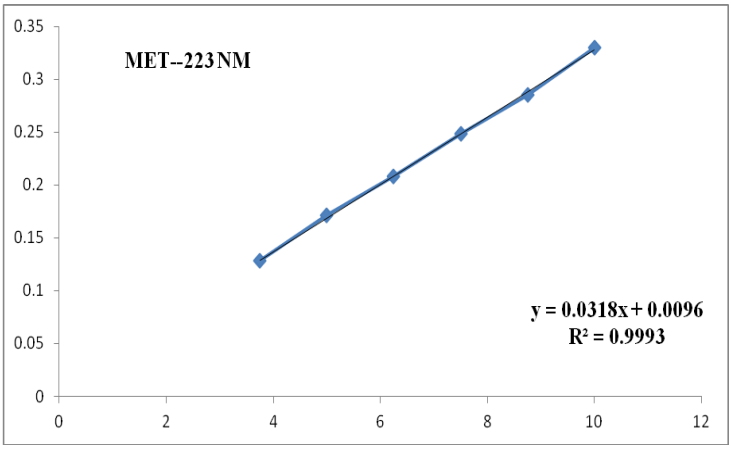


FIG. 8 CALIBRATION GRAPHS OF MET AND TEL BY ZERO ORDER ABSORBANCE CORRECTION METHOD

Method 4-

Combination of First derivative Dual Wavelength (MET) and zero crossing first derivative spectrophotometry (TEL): The absorption spectra of MET (3.75-10 µg/ml) and TEL (6-16 µg/ml) were recorded in the range of 220 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with Δλ = 4 nm and scaling factor 50 **(Fig. 9)**. At 330 nm, MET is having zero crossing point and TEL can be determined .The amplitudes at 330.0 nm were plotted against the respective concentrations of TEL for the preparation of calibration graph **(Fig. 10).**

The zero-order spectra of pure drugs of MET (3.75-10 µg/ml) and TEL (6-16 µg/ml) were derivatised in first order with Δλ = 4 nm and scaling factor 50 for both drugs **(Fig. 9)**. In this method, the difference between absorbance at 282.4 and 284.6 nm (Difference is zero TEL) of the 1st derivative spectra of the TEL were measured for determination of MET. For this difference of absorbance at 282.4 and 284.6 nm for MET were plotted against the respective concentrations of MET for the preparation of calibration graph **(Fig. 10)**.

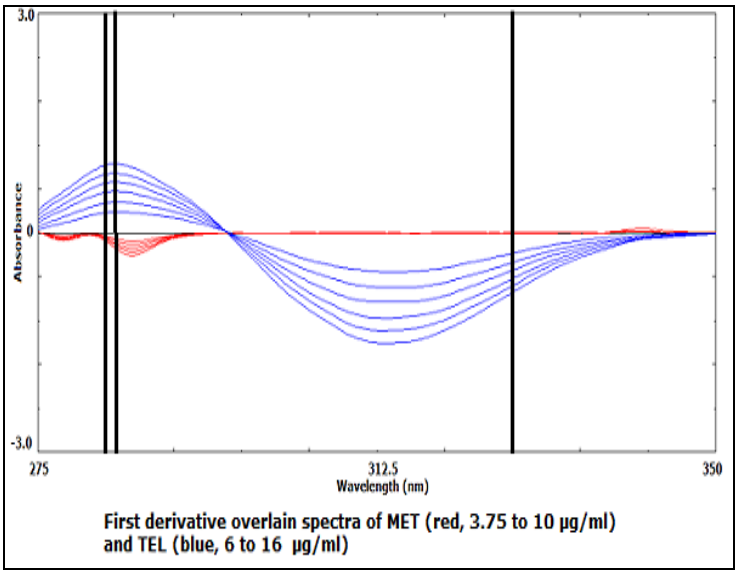
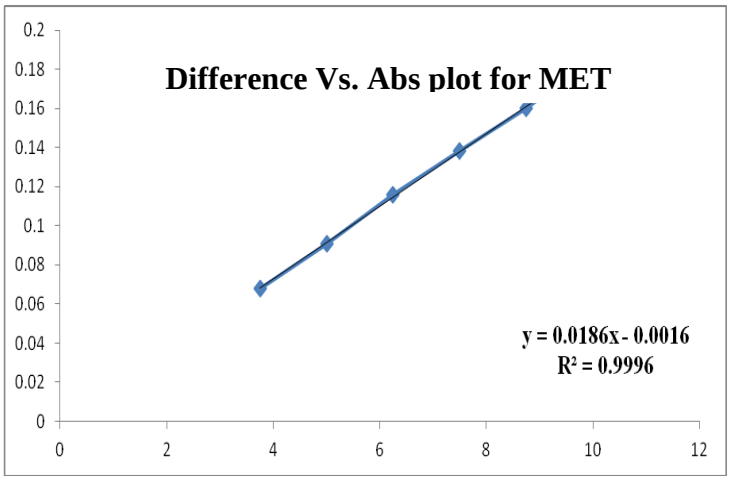


FIG. 9: FIRST DERIVATIVE OVERLAIN SPECTRA OF MET (3.75, 5, 6.25, 7.5, 8.75, 10 µg/ml, red) and TEL (6, 8, 10, 12, 14, 16 µg/ml, blue)



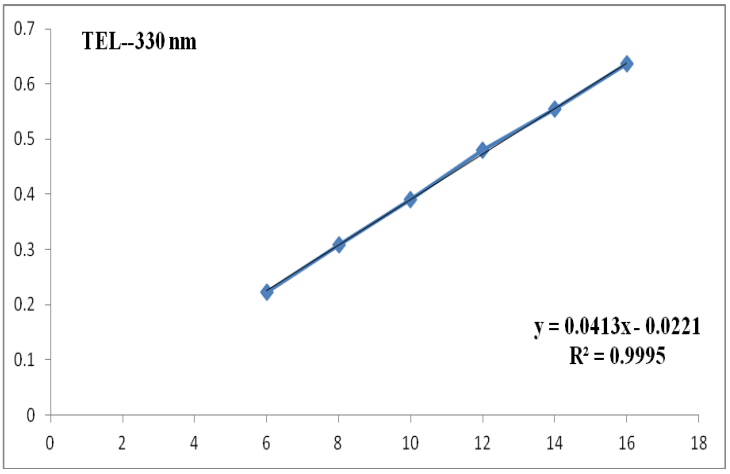


FIG. 10. CALIBRATION GRAPHS OF MET AND TEL BY FIRST ORDER DUAL WAVELENGTH (MET) AND ZERO CROSSING METHOD (TEL)

Assay of Commercial Formulation by Method by method 1, 2, 3 and 4: 20 tablets were powdered and an amount equivalent to 25 mg MET and 40 mg TEL was weighed and dissolved in 25 ml methanol. Solutions were filtered using whatmann filter paper grade 1. Appropriate dilutions were prepared in methanol taking suitable aliquots of the clear filtrates and subjected to analysis using all the four methods described above. The result of analysis is reported **(Table 1)**.

TABLE 1: RESULTS OF SIMULTANEOUS ESTIMATION OF MARKETED FORMULATION FOR METHOD 1, 2, 3 AND 4

Formulation :- TELSARBETA		
Labelled claim :- MET : TEL (25 mg : 40 mg)		
Method	**MET ***	**TEL ***
1	99.25 ± 0.68 %	98.75 ± 0.87 %
2	98.67 ± 0.85 %	99.16 ± 0.98 %
3	99.65 ± 0.22 %	99.36 ± 0.46 %
4	99.48 ± 0.36 %	99.44 ± 0.58 %

* Mean value of five determinations.

RESULTS AND DISCUSSION: Developed spectrophotometric methods for the simultaneous were validated according to ICH guidelines and data complying with the standards were obtained. The results of validation parameters for all the four developed methods are reported **(Table 2 and 3).**

TABLE 2: SUMMARY OF VALIDATION PARAMETERS BY DEVELOPED METHODS

Parameters	Method 1		Method 2		Method 3		Method 4	
	MET	TEL	MET	TEL	MET	TEL	MET	TEL
Analytical wavelength	230.2	237	231.8	237	223	296.6	282.4&284.6	330
Beer's range (µg/ml)	3-20	4-16	3-20	4-16	3-20	4-16	3-20	4-16
Correlation coefficient	0.9995	0.9991	0.9995	0.9991	0.9993	0.9996	0.9996	0.9995
Intraday precision (%RSD)	0.606	0.552	0.779	0.552	0.521	0.646	0.935	1.005
Interday precision (%RSD)	0.772	0.952	1.048	0.952	0.937	1.042	1.287	1.510
LOD (µg/ml)	1.15	1.05	0.98	1.12	0.75	1.25	1.28	1.10
LOQ (µg/ml)	3.45	3.15	2.94	3.36	2.25	3.75	3.84	3.30

TABLE 3: RESULTS OF RECOVERY STUDY OF TEL AND MET BY DEVELOPED METHODS

METHOD	% SPIKING	C_{ACTUAL}		C_{ADDED}		C_{FOUND} *		%RECOVERY	
		TEL	MET	TEL	MET	TEL	MET	TEL	MET
	80	6	3.75	4.8	3	10.70	6.72	99.07	99.56
1	100	6	3.75	6	3.75	11.98	7.56	99.83	100.80
	120	6	3.75	7.2	4.5	13.10	8.20	99.24	99.39
	80	6	3.75	4.8	3	10.76	6.88	99.65	101.99
2	100	6	3.75	6	3.75	11.96	7.66	99.63	102.11
	120	6	3.75	7.2	4.5	13.44	8.37	101.80	101.48
	80	6	3.75	4.8	3	10.79	6.71	99.88	99.43
3	100	6	3.75	6	3.75	11.97	7.44	99.78	99.26
	120	6	3.75	7.2	4.5	13.15	8.22	99.65	99.66
	80	6	3.75	4.8	3	10.78	6.70	99.79	99.24
4	100	6	3.75	6	3.75	11.96	7.40	99.70	98.64
	120	6	3.75	7.2	4.5	13.22	8.20	100.17	99.45

* Mean of three determinations

CONCLUSION: Four Spectrophotometric methods were developed for simultaneous estimation of MET and TEL in their combined formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of MET and TEL in marketed formulation.

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