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Development and Validation of UV Spectrophotometric Methods for Simultaneous Estimation of Trimetazidine hydrochloride and Metoprolol succinate in Tablet Dosage Form

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ABSTRACT:

A simple, accurate and precise First Order Derivative, Induced Dual Wavelength and Dual Wavelength Methods are developed for the simultaneous estimation of Trimetazidine hydrochloride (TMZ) and Metoprolol succinate (METO) in tablet dosage form. First Order Derivative method was developed using two wavelengths which are 243.93 nm (ZCP of METO) was used for quantification of TMZ and 268.92 nm (ZCP of TMZ) was used for quantification of METO. Induced Dual Wavelength was developed for TMZ shows similar equality factor (absorbance ratio=1.897) and difference absorbance at 270nm (λmax of TMZ) and 254nm, while METO shows similar equality factor (absorbance ratio=3.852) and difference absorbance at 274nm (λmax of METO) and 254nm. Dual Wavelength was developed TMZ shows similar absorbance at 280 and 260 nm for estimation of METO, while METO shows similar absorbance at 264.20 and 282.60 nm for estimation of TMZ. The linearity was established for above method over the concentration range of 40-200µg/ml for TMZ and 54-270µg/ml for METO with correlation coefficient R2 for First Order Derivative 0.9990 and 0.9996, for Induced Dual Wavelength 0.9995 and 0.9991 and for Dual Wavelength 0.9998 and 0.9993 respectively. Water was used as a solvent.
The results of the analysis were analyzed and validated statistically and recovery studies were carried out as per ICH guidelines. It can be used for routine analysis of two drugs in bulk as well as in pharmaceutical formulations.

KEY WORDS: Trimetazidine Hydrochloride (TMZ), Metoprolol Succinate (METO), First Order Derivative Method, Induced Dual Wavelength Method and Dual Wavelength Method.

INTRODUCTION[1-4]:

Trimetazidine Hydrochloride, chemically 1-(2, 3,4trimethoxybenzyl)-piperazine hydrochloride is used in Angina pectoris and powerful anti-ischemic agent. Metoprolol succinate, chemically Bis[(2RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1 methyl ethyl)amino]propan-2-ol] butanedioate is used in the treatment of Hypertension, angina. Clinically a combination is being used in the treatment of Hypertension and Angina for better therapeutic effect.

The combination of TMZ and METO is not official in any official pharmacopoeia. A literature survey revealed that two analytical methods were reported for the simultaneous estimation of TMZ and METO in Tablet dosage form. Hence in the present study a physical mixture of TMZ and METO was being taken for

simultaneous estimation by UV method. This present investigation describes a rapid, accurate and precise UV method of TMZ and METO in combination using Water as a solvent.

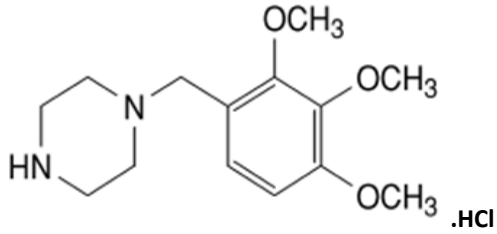

Figure: 1: Structure of Trimetazidine hydrochloride

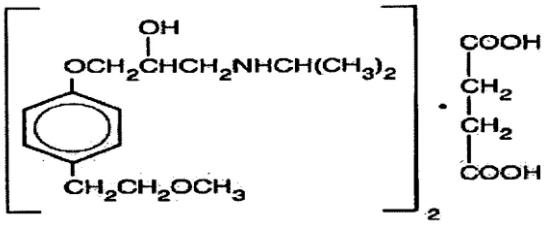

Figure: 2: Structure of Metoprolol succinate

MATERIALS AND METHODS:

Instruments and Apparatus:

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800, software – UV probe, version 2.42) with a pair of 1 cm matched quartz cells. All weighing was done on Reptech electronic analytical balance. All the apparatus used were Calibrated.

Reagents and Chemicals:

Trimetazidine hydrochloride (Gift sample, Triveni Pharmaceuticals, Vapi) and Metoprolol succinate (Gift sample, IPCA Pharmaceuticals, Vapi) and Distilled water.

Marketed Formulation:

Combined Tablet Formulation was purchased from Local market.

Preparation of Standard solution:

Trimetazidine Hydrochloride (TMZ) standard stock solution:

100 mg of TMZ standard was weighed and transferred to a 100 ml volumetric flask. The drug was dissolved by adding 25 ml of water and volume was made up to the mark with water to give a solution containing 1000 µg/ml TMZ. From this solution 10 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the water to give a solution containing 400µg/ml TMZ.

Metoprolol succinate (METO) standard stock solution:

100 mg of METO standard was weighed and transferred to a 100 ml volumetric flask. The drug was dissolved by adding 25 ml of water and volume was made up to the mark with water to give a solution containing 1000 µg/ml METO. From this solution 13.5 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the methanol to give a solution containing 540µg/ml METO.

Selection of Analytical Wavelength:

Standard 40-200µg/ml solutions of TMZ and 54-270µg/ml solution of METO were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm.

First order derivative method

All zero order spectrum (D0) were converted to first order derivative spectrum (D1) using delta lambda 2.0 and scaling factor 1. The overlain first order derivative spectrums of TMZ and METO at different concentration were recorded. The zero crossing point (ZCP) of TMZ was found to be 268.92nm and ZCP of METO was found to be 243.93 nm.

Induced dual wavelength method

The overlain zero order derivative spectrums of TMZ and METO at different concentration were recorded. TMZ shows similar equality factor (absorbance ratio=1.897) and difference absorbance at 270nm (λ_{max} of TMZ) and 254nm, while METO shows similar equality factor (absorbance ratio=3.852) and difference absorbance at 274nm (λ_{max} of METO) and 254nm.

Dual wavelength method

The overlain zero order derivative spectrums of TMZ and METO at different concentration were recorded. TMZ shows similar absorbance at 280nm and 260 nm for estimation of METO, while METO shows similar absorbance at 264.20nm and 282.60 nm for estimation of TMZ.

Method:

Calibration curve for the TMZ (40-200 µg/ml)

1, 2, 3, 4 and 5 ml volume of aliquot from standard TMZ stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the water to obtain concentration of 40, 80, 120, 160 and 200µg/ml respectively. The curve of each solution against the water was recorded. The straight-line equation was determined.

Calibration curve for the METO (54 - 270 µg/ml)

1, 2, 3, 4 and 5ml volume of aliquot from standard METO stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 54, 108, 162, 216 and 270µg/ml respectively. The curve of each solution against the water was recorded. The straight-line equation was determined.

Preparation of Sample solution:

Twenty tablets were weighed and finely powdered. The powder equivalent to 35 mg TMZ and 47.5 mg METO was accurately weighed and transferred to volumetric flask of 50ml capacity. Powder was dissolved in water in volumetric flask. The flask was shaken and volume was made up to the mark with water. The solution was filtered through whatmann filter paper (0.45µ). Appropriate volume of aliquot was taken and transferred to volumetric flask of 25 ml capacity to obtain a solution of 400µg/ml of TMZ and 540µg/ml of METO. Volume was made up to the mark with methanol. Further 3.0 ml of this solution was transferred to volumetric flask of 10ml capacity. Volume was made up to the mark with methanol to give a solution containing 120µg/ml TMZ and 162µg/ml METO. This solution was used for the estimation of TMZ and METO in tablet dosage form.

Estimation of TMZ and METO by First Order Derivative Method.

The First Order Derivative absorbance of each solution were measured at 243.93 nm (Zero crossing Point of METO) and 268.92 (Zero crossing Point of TMZ) for quantification of TMZ and METO. The amounts of the TMZ and METO present in the sample solution were calculated by fitting the responses into the regression equation for TMZ and METO in the proposed method.

For TMZ, Y= -0.0013X + 0.0303

For METO, Y= 0.0002X - 0.0012

Estimation of TMZ and METO by Induced Dual Wavelength Method.

The Zero Order Derivative spectrum, similar equality factor (absorbance ratio=F) of TMZ solutions were measured at 270nm (λ_{max} of TMZ) and 254nm, while METO solutions were measured at 274nm (λ_{max} of METO) and 254nm. Absorbance difference measured after multiplied TMZ and METO spectrum with similar equality factor of METO and TMZ respectively for quantification of TMZ and METO. The amounts of the TMZ and METO present in the sample solution were calculated by fitting the responses into the regression equation for TMZ and METO in the proposed method.

For TMZ, Y= 0.0030X + 0.1475

For METO, Y= 0.0059X + 0.0009

Estimation of TMZ and METO by Dual Wavelength Method.

The Zero Order Derivative spectrum, zero absorbance difference of TMZ solutions were measured at 280nm and 260nm, while METO were measured at 264.20nm and 282.60nm for quantification of METO and TMZ respectively. The amounts of the TMZ and METO present in the sample solution were calculated by fitting the responses into the regression equation for TMZ and METO in the proposed method.

For TMZ, Y= 0.0011X - 0.0007

For METO, Y= 0.0014X + 0.0065

Validation of spectrophotometric method:

1) Accuracy

Accuracy was determined by calculating recovery of TMZ and METO by the standard addition method. Known amounts of standard solutions of TMZ and METO were added to pre-quantified test solutions. Each solution was measured in triplicate, and the recovery was calculated by measuring absorbance for First order derivative and absorbance difference for Induced dual wavelength and Dual wavelength method.

(2) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

(3) Repeatability

First order derivative method

Standard solutions of TMZ were prepared of linearity range and spectrums were recorded. Absorbance was measured at 243.93 nm (ZCP of METO). In the similar manner solutions of METO were prepared and spectrums were recorded. Absorbance was measured at 268.92 nm (ZCP of TMZ). The procedure was repeated for six times and RSD was calculated.

Induced dual wavelength method

Standard solutions of TMZ were prepared of linearity range and spectrums were recorded. Absorbance difference was measured at 270 nm (λ_{max} of TMZ) and 254 nm after multiplied with similar equality factor of METO. In the similar manner solutions of METO were prepared and spectrums were recorded. Absorbance difference was measured at 274 nm (λ_{max} of METO) and 254 nm after multiplied with similar equality factor of TMZ. The procedure was repeated for six times and RSD was calculated.

Dual wavelength method

Standard solutions of TMZ were prepared of linearity range and spectrums were recorded. Absorbance difference was measured at 264.20 nm and 282.60 nm. In the similar manner solutions of METO were prepared and spectrums were recorded. Absorbance difference was measured at 280nm and 260 nm. The procedure was repeated for six times and RSD was calculated.

(4)Intraday and inter day precision

Variations of results within the same day (intraday), variation of results between days (interday) were analyzed.

First order derivative method

Intraday precision was determined by analyzing TMZ and METO individually for three times in same day, while Interday precision for three day at 243.93 nm (ZCP of METO) and 268.92 nm (ZCP of TMZ).

Induced dual wavelength method

Intraday precision was determined by analyzing TMZ and METO individually for three times in the same day, while Interday precision for three day at 270 nm (λ_{max} of TMZ) and 254 nm absorbance difference for TMZ after multiplied with similar equality factor of METO and similar at 274 nm (λ_{max} of METO) and 254 nm for METO after multiplied with similar equality factor of TMZ.

Dual wavelength method

Intraday precision was determined by analyzing TMZ and METO individually for three times in the same day, while Inter day precision for three day at 264.20 nm and 282.60 nm absorbance difference for TMZ and similar 280 nm and 260 nm absorbance difference for METO.

(5) Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

RESULT AND DISCUSSION:

First order derivative method

A reliable first order derivative spectrophotometric method was developed for simultaneous estimation of TMZ and METO in combined dosage form by UV Spectrophotometry. Calibration data at 243.93 nm (ZCP of METO) and 268.92 nm (ZCP of TMZ) for TMZ and METO are shown in Table. Calibration curves for TMZ and METO were plotted between absorbance and concentration. The following equations for straight line were obtained for TMZ and METO.

Linear equation for TMZ at 243.93 nm (ZCP of METO), **Y= -0.0013X + 0.0303**

Linear equation for METO at 268.92 nm (ZCP of TMZ), **Y= 0.0002X - 0.0012**

The developed First Order Derivative Method was validated. The linear range, correlation coefficient, detection limit and standard deviation for TMZ and METO by Spectroscopy method are shown in Table. Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 98.15-101.68 %. Precision was calculated as repeatability and intraday and interday variation for two drugs. The LOD and LOQ for TMZ were found to be 1.53µg/ml and 4.65µg/ml at 243.93 nm (ZCP of METO). The LOD and LOQ for METO were found to be 9.76µg/ml and 29.60µg/ml at 268.92 nm (ZCP of TMZ) respectively. Summary of validation parameters are shown.

Marketed formulation was analyzed by the proposed

method and assay result of marketed formulation is shown.

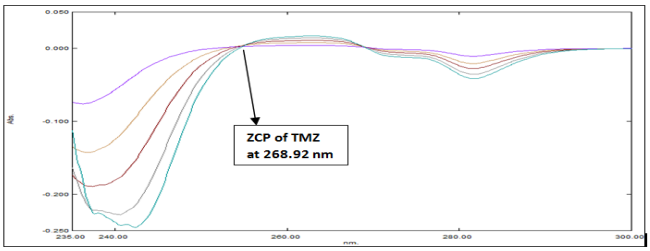

Figure: 3: First Order Spectra of TMZ (40-200 µg/ml)

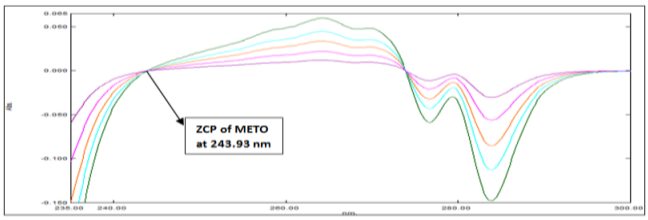

Figure: 4: First Order Spectra of METO (54-270 µg/ml)

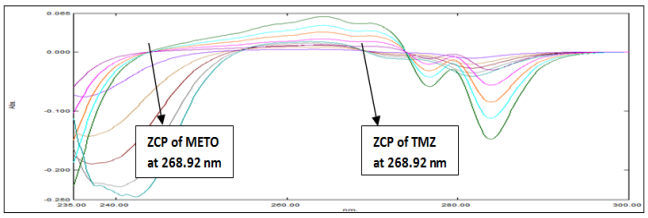

Figure: 5: Overlain First Order Spectra of TMZ and METO.

Linearity of TMZ at 243.93 nm (ZCP of METO)

Table: 1: Result of calibration readings of TMZ

Sr. No.	Concentration (µg/ml)	Derivative Value of TMZ at 243.93 nm Mean ± S.D. (n=6)
1.	40	-0.0243 ± 0.0005
2.	80	-0.0700 ± 0.0004
3.	120	-0.1230 ± 0.0007
4.	160	-0.1796 ± 0.0005
5.	200	-0.2281 ± 0.0007

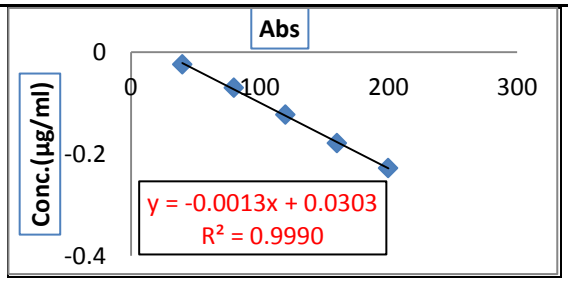

Figure: 6: Calibration curve of TMZ at 243.93 nm (ZCP of METO)

Linearity of METO at 268.92 nm (ZCP of TMZ)

Table: 2: Result of calibration readings of METO

Sr. No.	Concentration (µg/ml)	Derivative Value of METO at 268.92 nm Mean ± S.D. (n=6)
1.	54	0.0091 ± 0.0004
2.	108	0.0180 ± 0.0008
3.	162	0.0283 ± 0.0005
4.	216	0.0386 ± 0.0008
5.	270	0.0481 ± 0.0004

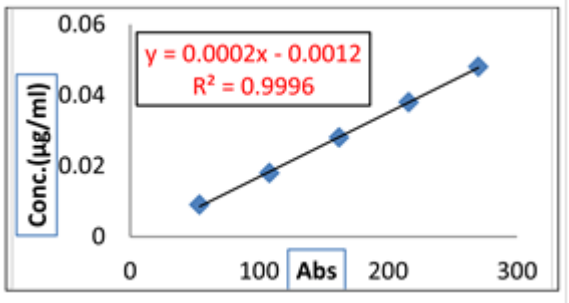

Figure: 7: Calibration curve of METO at 268.92 nm (ZCP of TMZ)

PRECISION:-

1. Repeatability: - The repeatability of TMZ at 243.93nm and METO at 268.92 nm is shown in table.

Table: 3: Result of Repeatability Study for TMZ and METO

Drugs	Concentration (µg/ml)	Mean abs. ± S.D. (n=6)	%R.S.D.
TMZ	120	-0.1231 ± 0.0005	0.4209
METO	162	0.0287 ± 0.0005	0.9682

2. Intraday Precision and Interday Precision: - The data of intraday precision and interday precision for TMZ at 243.93 nm (ZCP of METO) and METO at 268.92 nm (ZCP of TMZ) is shown in table.

Table: 4: Result of Intraday Precision and Interday Precision Study for TMZ and METO.

Drugs	Concentration (µg/ml)	Intraday data		Interday data	
		Mean abs. ± S.D. (n=3)	%R.S.D.	Mean abs. ± S.D. (n=3)	%R.S.D.
TMZ	80	-0.0696 ± 0.0005	0.8327	-0.0693 ± 0.0005	0.8327
	120	-0.1226 ± 0.0005	0.4719	-0.1210 ± 0.0010	0.8264
	160	-0.1796 ± 0.0005	0.3214	-0.1793 ± 0.0005	0.3219
METO	108	0.0179 ± 0.0003	1.7891	0.0178 ± 0.0003	1.8025

	162	0.0279 ± 0.0004	1.5623	0.0276 ± 0.0004	1.6603
	216	0.0386 ± 0.0004	1.1661	0.0385 ± 0.0006	1.5584

ACCURACY STUDY: - The accuracy of TMZ at 243.93 nm and METO at 268.92 nm is shown in table.

Table: 5: Result of Accuracy Study for TMZ and METO.

Drugs	Level	Amount of sample (µg/ml)	Amount of Std. Spiked (µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	%Recovery ± S.D. (n=3)
TMZ	0	40	0	40	39.20	-
	80	40	32	72	71	98.61 ± 1.0144
	100	40	40	80	78.95	98.68 ± 0.5570
	120	40	48	88	86.89	98.74 ± 0.5051
METO	0	54	0	54	54.33	-
	80	54	43	97	96	98.97 ± 0.0001
	100	54	54	108	106	98.15 ± 0.0001
	120	54	65	119	121	101.68 ± 0.0001

Assay Result of Marketed Formulation

Brand Name	Actual Concentration (µg/ml)		Amount Obtained Mean (µg/ml)		% Assay TMZ ± S.D. (n=3)	% Assay METO ± S.D. (n=3)
Carvidon - MT	TMZ	METO	TMZ	METO		
	120	162	120.23	161.00	100.19 ± 0.5444	99.38 ± 0.7071

Parameters of First Order Derivative Method

Parameters	TMZ (ZCP of METO)	METO (ZCP of TMZ)
Concentration Range	40-200 µg/ml	54-270 µg/ml
Regression equation	-0.0013x + 0.0303	0.0002x - 0.0012
Slope (m)	-0.0013	0.0002
Intercept (c)	0.0303	-0.0012
Regression co-efficient	0.9995	0.9998
LOD	1.53 µg/ml	9.76 µg/ml
LOQ	4.65 µg/ml	29.60 µg/ml
Repeatability(n=6) (%RSD)	0.4209	0.9682
Intraday precision (n=3) (% RSD)	0.3214-0.8327	0.3219-0.8327
Interday precision (n=3) (% RSD)	1.1661-1.7891	1.5584-1.8025
% Recovery	98.61-98.74%	98.15-101.68%

Induced dual wavelength method

A reliable Induced dual wavelength method was developed for simultaneous estimation of TMZ and METO in tablet dosage form by UV Spectrometry. Calibration data for TMZ absorbance difference at 270 nm (λmax of TMZ) and 254 nm, while for METO at 274 nm (λmax of METO) and 254 nm are shown in Table. Calibration curves for TMZ and METO were plotted between absorbance difference and concentration. The following equations for straight line were obtained for TMZ and METO.

Linear equation for TMZ at 270 nm and 254 nm, Y= 0.0030X + 0.1475

Linear equation for METO at 274 nm and 254 nm, Y= 0.0059X + 0.0009

The developed Induced dual wavelength method was validated. The linear range, correlation coefficient, detection limit and standard deviation for TMZ and METO by Spectroscopy method are shown in Table. Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 99.30-100.95 %. Precision was calculated as repeatability and intraday and interday variation for two drugs. The LOD and LOQ for TMZ were found to be 2.91µg/ml and 8.84µg/ml at 270 nm and 254 nm. The LOD and LOQ for METO were found to be 4.55µg/ml and 13.79µg/ml at 274 nm and 254 nm respectively. Summary of validation parameters are shown.

Marketed formulation was analyzed by the proposed method shown in assay result.

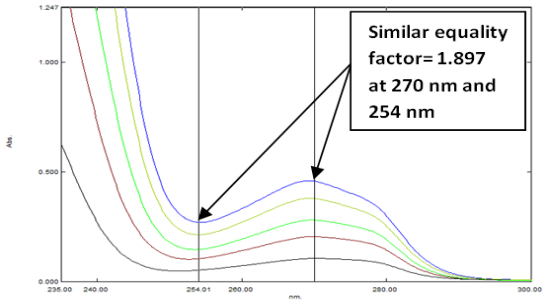

Figure: 8: Zero order Spectra of TMZ (40-200 µg/ml)

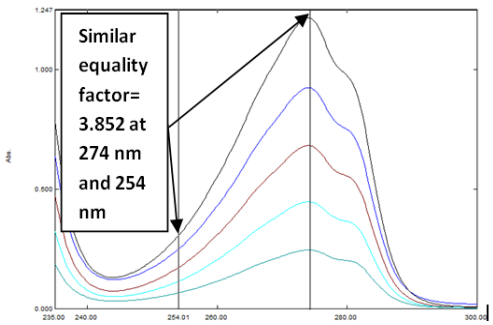

Figure: 9: Zero order Spectra of METO (54-270 µg/ml)

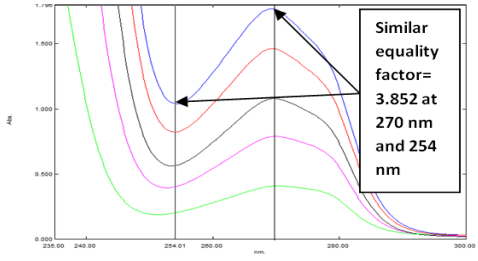

Figure: 10: Zero order Spectra of TMZ (40-200 µg/ml) after multiplied with equality factor of METO

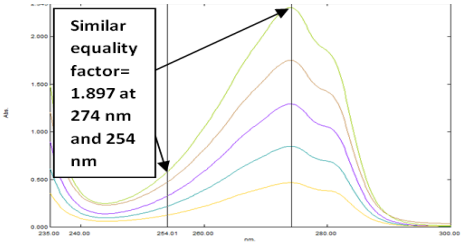

Figure: 11: Zero order Spectra of METO (54-270 g/ml) after multiplied with equality factor of TMZ

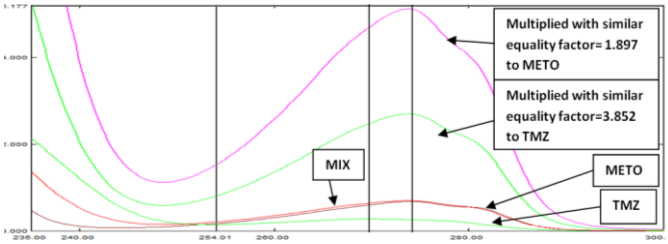

Figure: 12: Zero Order Spectra of TMZ (120 µg/ml), METO (162 µg/ml) and MIX

Linearity of TMZ at 270 nm and 254 nm

Table: 6: Result of calibration readings of TMZ

Sr. No.	Concentration (µg/ml)	Absorbance difference of TMZ at 270 nm and 254 nm after multiplied by 3.852 Mean ± S.D. (n=6)
1.	40	0.267 ± 0.0020
2.	80	0.388 ± 0.0008
3.	120	0.514 ± 0.0011
4.	160	0.637 ± 0.0005
5.	200	0.748 ± 0.0038

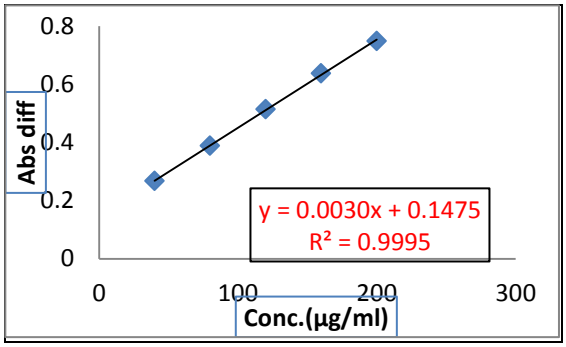

Figure: 13: Calibration curve of TMZ

Linearity of METO at 274 nm and 254 nm

Table: 7: Result of calibration readings of METO

Sr. No.	Concentration (µg/ml)	Absorbance difference of METO at 274 nm and 254 nm after multiplied by 1.897 Mean ± S.D. (n=6)
1.	54	0.337 ± 0.0005
2.	108	0.624 ± 0.0012
3.	162	0.942 ± 0.0037
4.	216	1.267 ± 0.0017
5.	270	1.606 ± 0.0034

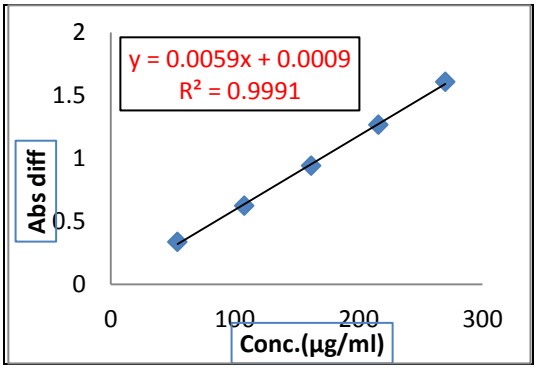

Figure: 14: Calibration curve of METO

PRECISION:-

1. Repeatability: - The data of repeatability for TMZ at 270 nm and 254 nm and METO at 274 nm and 254 nm is shown in table.

Table: 8: Result of Repeatability Study for TMZ and METO.

Drugs	Concentration (µg/ml)	Mean abs. diff. ± S.D. (n=6)	%R.S.D.
TMZ	120	0.514 ± 0.0005	0.1005
METO	162	0.942 ± 0.0011	0.1239

2. Intraday Precision and Interday Precision: - The data of intraday precision and interday precision for TMZ at

270nm and 254 nm and METO at 274 nm and 254 nm is shown in table.

Table: 9: Result of Intraday Precision Study for TMZ and METO

Drugs	Concentration (µg/ml)	Intraday data		Interday data	
		Mean abs. diff ± S.D. (n=3)	% R.S.D.	Mean abs. diff ± S.D. (n=3)	% R.S.D.
TMZ	80	0.387 ± 0.0005	0.1490	0.387 ± 0.0010	0.2583
	120	0.515 ± 0.0005	0.1121	0.512 ± 0.0020	0.3906
	160	0.636 ± 0.0005	0.0907	0.636 ± 0.0011	0.1814
METO	108	0.625 ± 0.0017	0.2771	0.624 ± 0.0020	0.3334
	162	0.945 ± 0.0010	0.1058	0.942 ± 0.0037	0.4017
	216	1.267 ± 0.0010	0.0789	1.263 ± 0.0015	0.1208

ACCURACY STUDY: - The data of accuracy for TMZ at 270nm and 254 nm and METO at 274 nm and 254 nm is shown in table

Table: 10: Result of Accuracy Study for TMZ and METO.

Drugs	Level	Amount of sample (µg/ml)	Amount of Std. Spiked (µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	%Recovery ± S.D. (n=3)
TMZ	0	40	0	40	39.72	-
	80	40	32	72	72.50	100.69 ± 0.4629
	100	40	40	80	80.61	100.76 ± 0.4811
	120	40	48	88	88.83	100.95 ± 0.5786
METO	0	54	0	54	54.42	-
	80	54	43	97	97.70	100.72 ± 0.2017
	100	54	54	108	107.31	99.36 ± 0.5658
	120	54	65	119	119.17	100.14 ± 0.0815

Assay Result of Marketed Formulation

Brand Name	Actual Concentration (µg/ml)		Amount Obtained Mean (µg/ml)		%Assay TMZ ± S.D. (n=3)	%Assay METO ± S.D. (n=3)
Carvidon-MT	TMZ 120	METO 162	TMZ 120.83	METO 161.2	100.69 ± 0.1617	99.51 ± 0.0577

Parameters of Induced dual wavelength Method

Parameters	TMZ (ZCP of METO)	METO (ZCP of TMZ)
Concentration Range	40-200 µg/ml	54-270 µg/ml
Regression equation	0.0030x + 0.1475	0.0059x + 0.0009
Slope (m)	0.0030	0.0059
Intercept (c)	0.1475	0.0009
Regression co-efficient	0.9997	0.9995
LOD	2.91 µg/ml	4.55 µg/ml
LOQ	8.84 µg/ml	13.79 µg/ml
Repeatability(n=6) (%RSD)	0.1005	0.1239
Intraday precision (n=3) (% RSD)	0.0907-0.1490	0.0789-0.2771
Interday precision (n=3) (% RSD)	0.1814-0.3906	0.1208-0.4017
% Recovery	100.69-100.95%	99.36-100.72%

Dual wavelength method

A reliable Dual wavelength spectrophotometric method was developed for simultaneous estimation of TMZ and METO in combined dosage form by UV Spectrophotometry. Calibration data for TMZ absorbance difference at 264.20 nm and 282.60 nm (where similar absorbance of METO), while for METO absorbance difference at 280 nm and 260 nm (where similar absorbance of TMZ) are shown in Table. Calibration curves for TMZ and METO were plotted between absorbance difference and concentration. The following equations for straight line were obtained for TMZ and METO.

Linear equation for TMZ at 264.20 nm and 282.60 nm, Y= 0.0011X - 0.0007
Linear equation for METO at 280 nm and 260 nm, Y= 0.0014X + 0.0065

The developed Dual wavelength method was validated. The linear range, correlation coefficient, detection limit and standard deviation for TMZ and METO by Spectroscopy method are shown in Table. Accuracy was determined by calculating the recovery. The method was

found to be accurate with % recovery 99.21-100.42 %. Precision was calculated as repeatability and intraday and interday variation for two drugs. The LOD and LOQ for TMZ were found to be 0.73µg/ml and 2.21µg/ml at 264.20 nm and 282.60 nm. The LOD and LOQ for METO were found to be 2.98µg/ml and 9.05µg/ml at 280 nm and 260 nm respectively. Summary of validation parameters are shown.

Marketed formulation was analyzed by the proposed method shown assay result.

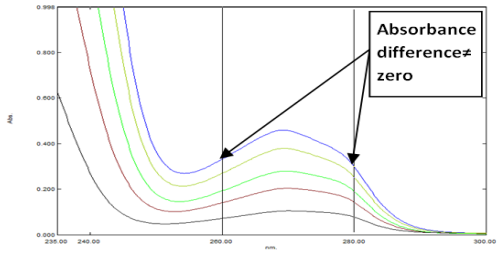

Figure: 15: Zero order Spectra of TMZ (40-200 µg/ml) at 280 nm and 260 nm

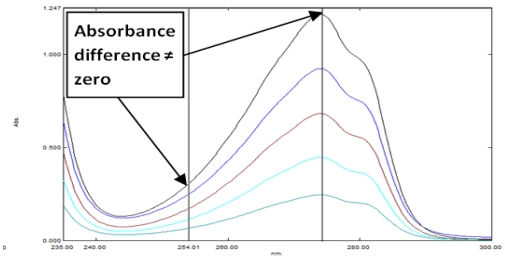

Figure: 16: Zero order Spectra of METO (54-270 µg/ml) at 264.20 nm and 282.60 nm

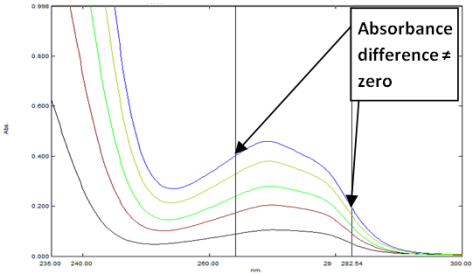

Figure: 17: Zero order Spectra of TMZ (40-200 µg/ml) at 264.20 nm and 282.60 nm

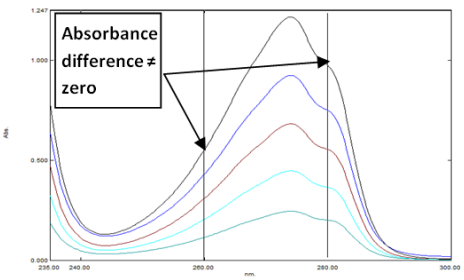

Figure: 18: Zero order Spectra of METO (54-270 µg/ml) at 280 nm and 260 nm

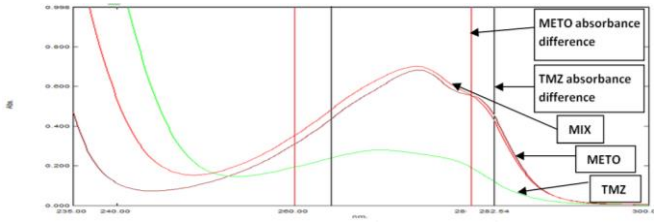

Figure: 19: Zero Order Spectra of TMZ (120 µg/ml), METO (162 µg/ml) and Mix

Linearity of TMZ at 264.20 nm and 282.60 nm

Table: 11: Result of calibration readings of TMZ

Sr. No.	Concentration (µg/ml)	Absorbance difference of TMZ at 264.20 nm and 282.60 nm Mean ± S.D. (n=6)
1.	40	0.040 ± 0.0004
2.	80	0.085 ± 0.0004
3.	120	0.128 ± 0.0011
4.	160	0.168 ± 0.0054
5.	200	0.216 ± 0.0075

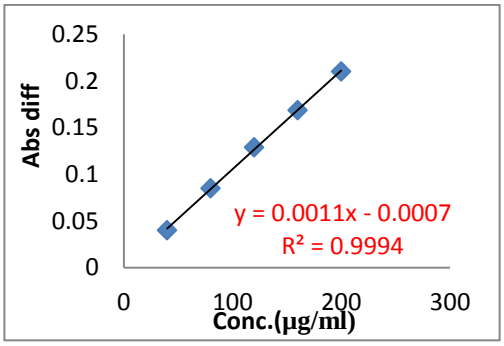

Figure: 20: Calibration curve of TMZ at 264.20 nm and 282.60 nm

Linearity of METO at 280 nm and 260 nm

Table: 12: Result of calibration readings of METO

Sr. No.	Concentration (µg/ml)	Absorbance difference of METO at 280 nm and 260 nm Mean ± S.D. (n=6)
1.	54	0.086 ± 0.0004
2.	108	0.160 ± 0.0005
3.	162	0.237 ± 0.0033
4.	216	0.317 ± 0.0012
5.	270	0.395 ± 0.0014

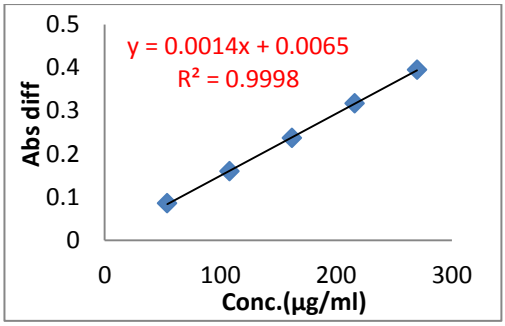

Figure: 21: Calibration curve of METO at 280 nm and 260 nm

PRECISION:-

1. Repeatability: - The data of repeatability for TMZ at 264.20 nm and 282.60 nm and METO at 280 nm and 260 nm is shown in table.

Table: 13: Result of Repeatability Study for TMZ and METO.

Drugs	Concentration (µg/ml)	Mean abs. diff. ± S.D. (n=6)	%R.S.D.
TMZ	120	0.129 ± 0.0005	0.3992
METO	162	0.237 ± 0.0005	0.2182

2. Intraday Precision: - The data of intraday precision for TMZ at 270nm and 254 nm and METO at 274 nm and 254 nm is shown in table.

Table: 14: Result of Intraday Precision and Interday Precision Study for TMZ and METO

Drugs	Concentration (µg/ml)	Intraday data		Interday data	
		Mean abs. diff ± S.D. (n=3)	% R.S.D.	Mean abs. diff ± S.D. (n=3)	% R.S.D.
TMZ	80	0.084 ± 0.0005	0.6864	0.008 ± 0.0010	1.176 5
	120	0.129 ± 0.0005	0.4464	0.129 ± 0.0010	0.775 2
	160	0.169 ± 0.0005	0.3409	0.168 ± 0.0020	1.241 6
METO	108	0.159 ± 0.0005	0.3623	0.157 ± 0.0025	1.599 0
	162	0.237 ± 0.0005	0.2439	0.235 ± 0.0015	0.648 1
	216	0.317 ± 0.0005	0.1819	0.315 ± 0.0020	0.660 1

ACCURACY STUDY: - The data of accuracy for TMZ at 264.20 nm and 282.60 nm and METO at 280 nm and 260 nm is shown in table.

Table: 15: Result of Accuracy Study for TMZ and METO.

Drugs	Level	Amount of sample (µg/ml)	Amount of Std. Spiked (µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	%Recovery ± S.D. (n=3)
TMZ	0	40	0	40	40.33	-
	80	40	32	72	71.85	99.79 ± 1.4579
	100	40	40	80	80.03	100.04 ± 1.3121
	120	40	48	88	87.30	99.21 ± 1.5780
METO	0	54	0	54	54.42	-
	80	54	43	97	97.70	100.03 ± 1.1283
	100	54	54	108	107.31	100.42 ± 1.0102
	120	54	65	119	119.17	99.94 ± 0.6002

Assay Result of Marketed Formulation

Brand Name	Actual Concentration (µg/ml)		Amount Obtained Mean (µg/ml)		%Assay TMZ ± S.D. (n=3)	%Assay METO ± S.D. (n=3)
Carvidon - MT	TMZ	METO	TMZ	METO		
	120	162	119.73	162.5	99.77 ± 0.8775	100.31 ± 0.2540

ANOVA test for developed and validated method

For the comparison of First order derivative Spectrophotometric method, Induced dual wavelength method and Dual wavelength method for estimation of TMZ and METO in tablet dosage form ANOVA was applied using Microsoft Excel-2008 data analysis tool.

Parameters of Induced dual wavelength Method

Parameters	TMZ (ZCP of METO)	METO (ZCP of TMZ)
Concentration Range	40-200 µg/ml	54-270 µg/ml
Regression equation	0.0011x - 0.0007	0.0014x + 0.0065
Slope (m)	0.0011	0.0014
Intercept (c)	-0.0007	0.0065
Regression co-efficient	0.9996	0.9998
LOD	0.73 µg/ml	2.98 µg/ml
LOQ	2.21 µg/ml	9.05 µg/ml
Repeatability(n=6) (%RSD)	0.3992	0.2182
Intraday precision (n=3) (% RSD)	0.3409-0.6864	0.1819-0.3623
Interday precision (n=3) (% RSD)	0.7752-1.2416	0.6481-1.5990
% Recovery	99.21-100.04%	99.94-100.42%

Table: 16: Assay results of developed methods for TMZ (µg/ml).

Sr. No.	First Order Derivative method	Induced dual wavelength method	Dual wavelength Method
1.	120.23	120.83	119.73
2.	121.00	120.83	119.73
3.	119.46	121.17	121.54

Table: 17: ANOVA for TMZ assay.

Source of Variation	SS	df	MS	F calculate	F critical
Between Group	0.8916	2	0.4458	0.7760	5.1432
Within Group	3.4469	6	0.5744		

Table: 18: Assay results of developed method for METO (µg/ml).

Sr. No.	First Order Derivative method	Induced dual wavelength method	Dual wavelength Method
1.	161.00	161.37	162.50
2.	166.00	161.20	162.50
3.	161.00	161.37	163.21

Table: 19: ANOVA for METO assay

Source of Variation	SS	df	MS	F calculate	F critical
Between Group	3.8622	2	1.9311	0.6806	5.1432
Within Group	17.022	6	2.837		

The calculated F value is lower than that of tabulated F value, indicating no significant difference is observed in assay results obtained by all the three methods for both drugs.

CONCLUSION:

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. The low value of relative standard deviation for repeated measurement indicates that the method is precise. The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage forms without any interference due to the other components present in the formulations.

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