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"Development and Validation of UV Spectroscopic Methods for Simultaneous Estimation of Clomiphene Citrate and Melatonin in Marketed Formulation"

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ABSTRACT
A fixed dose combination of CLOMIPHENE CITRATE & MELATONIN is used in ratio of 50 mg: 3 mg as tablet for treatment of female infertility. A simple, precise, accurate and economical UV spectrophotometric First Order Derivative Spectroscopy and Absorbance Correction Method were developed and validated for Quantitative determination of CLOMIPHENE CITRATE & MELATONIN in Marketed Formulation. First order derivative spectrophotometric method two Zero Crossing Points (ZCP) were selected as 234.5 nm for Clomiphene citrate (CLP) and 249 nm for Melatonin (MLT). Estimation of CLP was done at 249 nm (ZCP of MLT) and MLT at 234.5 nm (ZCP of CLP) respectively. Absorbance Correction Method, absorbance taken at two selected wavelengths, CLP was estimated directly at 324 nm and MLT was estimated at 225 nm (Absorbance deduction) were CLP have some Absorbance. UV Spectrophotometric method for CLP & MLT was found to be linear over the range of 20-60 µg/ml and 1-5 µg/ml respectively for First order derivative spectroscopic method and Absorbance correction method.

Key Words:
Clomiphene Citrate, Melatonin, First Order Derivative Spectroscopic Method, Absorbance Correction Method

I. INTRODUCTION

In ultraviolet spectroscopy, the absorption or reflectance of the electromagnetic spectrum in the ultraviolet and nearby visible regions is studied. It is also known as UV-Vis, or UV-visible spectrophotometry. This methodology's inexpensive cost and ease of implementation make it widely used in a variety of fundamental and practical applications. All that is required for the sample to be considered a chromophore is for it to absorb in the UV-Vis region. Not only is fluorescence spectroscopy employed, but also absorption spectroscopy. The important variables are wavelength, absorbance, transmittance (%T), and reflectance (%R), together with the variations in each over time. clomiphene citrate (2-[p-(2-chloro-1,2-diphenylvinyl) phenoxy] triethylamine citrate) it is Freely soluble in methanol, soluble in ethanol, slightly soluble in acetone, water, and chloroform, and insoluble in ether and melatonin (N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide) it is Soluble in water (0.1 mg/ml), ethanol (8 mg/ml), benzene, chloroform, methanol, DMSO, toluene, and dilute aqueous acid, and very slightly soluble in petroleum ether. For women who are not ovulating and are unable to become pregnant, it is effective. Female infertility is treated with a combination of melatonin and clomiphene citrate. For women who are not ovulating and are unable to become pregnant, it is effective.

II. MATERIALS AND METHODS

- **APPARATUS AND INSTRUMENT**

UV-Visible Spectrophotometer (Double Beam) (Shimadzu-1900i, Software- Lab solution) having matched Quartz cells of light path 1 cm, Weighing balance (Electronic analytical balance)- (Shimadzu-0.1mg), Ultra-Sonicator (Athena Technology), Volumetric Flask – 10 ml (Borosil), Pipettes- 1,5 ml (Borosil)

- **REAGENTS AND MATERIALS**

Clomiphene citrate and Melatonin (gift sample from Akums Drug Pharmaceutical Pvt. Ltd., Delhi.), Methanol (UV Grade – Thomas Baker, Mumbai).

➢ **SPECTROPHOTOMETRIC CONDITION**

- Mode: Scan

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- Scan Speed: Medium
- Wavelength range: 200 - 400 nm
- Scale of Absorbance: 0.00 - 2.00 A
- Base line correction: Methanol

➢ SELECTION OF SOLVENT

Common solvent for both drugs was found to be methanol as per solubility study. Therefore, methanol was selected as solvent for UV methods. In methanol, drugs (Clomiphene Citrate and Melatonin) give linear spectra at their measured wavelength. So, Methanol is the preferred solvent.

➢ PREPARATION OF STANDARD SOLUTION

1. Preparation of CLP standard stock solution (1000 µg/ml):

10 mg of CLP was weighed and transferred to 10 ml volumetric flask. It was dissolved in methanol and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml. Aliquot of 2.5 ml from above standard stock solution was pipetted out into 25 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing 100 µg/ml.

2. Preparation of MLT standard stock solution (1000 µg/ml):

10 mg of MLT was weighed and transferred to 10 ml volumetric flask. It was dissolved in methanol and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml. Aliquot of 1 ml from above standard stock solution was pipetted out into 10 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing 100 µg/ml. Aliquot of 2.5 ml from above working stock solution (100 µg/ml) was pipetted out into 25 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing 10 µg/ml.

❖ FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

➢ SELECTION OF WAVELENGTH

Aliquots of 5 ml from working stock solution of CLP (100 µg/ml) and 3 ml from working stock solution of MLT (10 µg/ml) were pipette out and taken into two separate volumetric flasks of 10 ml and volume was made up to mark with methanol to give a solution containing 50 µg/ml and 3 µg/ml of CLP and MLT. Each solution was scanned between 200-400 nm against methanol as blank. The zero order spectra data was processed to obtain first order derivative spectrum in the range of 400-200 nm. In first order spectra Zero Crossing Point for CLP 234.5 nm and 249 nm for MLT. Wavelength selected for quantitation were 249 nm for CLP (ZCP of MLT) and 234.5 nm for MLT (ZCP of CLP).

➢ PREPARATION OF CALIBRATION CURVE

1. Calibration curve for CLP

Calibration curve for CLP consisted of five different concentrations of standard solution of CLP ranging from 20-60 µg/ml. The First derivative (d_A/d_λ) spectra of all these solutions were obtained by transformation of zero order spectra of every solution. d_A/d_λ absorbance at 249 nm (Zero Crossing Point of MLT) was computed and the plot of d_A/d_λ absorbance vs. concentration was plotted and regression equation was obtained.

2. Calibration curve for MLT

Calibration curve for MLT consisted of five different concentrations of standard solution of MLT ranging from 1-5 µg/ml. The First derivative (d_A/d_λ) spectra of all these solutions were obtained by transformation of zero order spectra of every solution. d_A/d_λ absorbance at 234.5 nm (Zero Crossing Point of CLP) was computed and the plot of d_A/d_λ absorbance vs. concentration was plotted and regression equation was obtained.

➢ VALIDATION OF METHODS

Parameters to be considered for the validation of method are:

1. Linearity

The linearity response was determination by analyzing 5 independent levels of calibration curve in the range of 20-60 µg/ml for CLP, 1-5 µg/ml for MLT (n=5).

2. Precision

A. Repeatability

Repeatability of Developed method was assessed by analyzing50 µg/ml, 3 µg/ml of CLP and MLT solution was six times (n=6) and % R.S.D. was calculated.

B. Intraday precision (n=3)

Intraday Precision assessed analyzing by 40, 50 and 60 µg/ml of CLP and 2, 3 and 4 µg/ml of MLT of same batch Solution for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

C. Interday Precision (n=3)

Interday Precision assessed analyzing by 40, 50 and 60 µg/ml of CLP and 2, 3 and 4 µg/ml of MLT of same batch Solution for three times (n=3) on three different days and % R.S.D. was calculated.

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3. Accuracy (n=3)

Tablet mixture of 50 mg equivalent of CLP was taken into 10 ml of volumetric flask. Methanol was added and sonicated for 2-3 mins and volume was made up to mark with methanol. Filtered the through Whatman filter paper no. 42. Thus, resulting solution gave µg/ml of CLP and 1000 µg/ml of MLT. From the above solution, 1.0 ml was pipette out and transferred to 10 ml volumetric flask and volume was made up to mark with methanol in order to give a solution containing CLP (50 µg/ml) + MLT (3 µg/ml).The amount of CLP and MLT was calculated at each level (80%, 100% and 120%) and % recoveries were computed.

4. LOD and LOQ

The LOD (Limit of Detection) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula:

LOD = 3.3 × S.D./Slope

Where, S.D. = Standard deviation of the Y-intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

The LOQ (Limit of Quantitation) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula:

LOQ=10×S.D./Slope

Where, S.D. = Standard deviation of the Y-intercepts of 5 calibration curves

APPLICABILITYOF FIRST ORDER DERIVATIVE METHOD

Tablet powder equivalent to about 50 mg CLP, 3 mg MLT was transferred to 100 ml volumetric flask, methanol was added and sonicate for 10-15 min, volume was than make up to the mark with methanol (500 µg/ml of CLP and 30 µg/ml MLT) and the solution filtered through Whatman filter paper No.41. This solution was use at stock solution 2 ml of aliquot solution was pipetted out and transferred to a 10 ml volumetric flask. Then the volume made up to the mark with methanol (100 µg/ml of CLP and 6 µg/ml of MLT). Then 5 ml was withdrawn from above working solution and transferred into 10 ml volumetric flask. Then the volume was made up to mark with methanol to get sample solution containing 50 µg/ml of CLP and 3 µg/ml of MLT respectively. Absorbance ofresultingsolutionwasrecordedbyconvertingzeroorderspectraintofirstorderat249 nm for CLP (ZCP of MLT) and 234.5 nm for MLT (ZCP of CLP). The concentration of CLP and MLT was obtained by solving the regression equation:

1.y =0.0011x +0.0012 for CLP at 249nm (ZCP of MLT)

2.y =0.0101x + 0.0011 for MLT at 234.5nm (ZCP of CLP)

APPLICABILITY OF ABSORBANCE CORRECTION METHOD

Tablet powder equivalent to about 50 mg CLP, 3 mg MLT was transferred to 100 ml volumetric flask, methanol was added and sonicate for 10-15 min, volume was than make up to the mark with methanol (500 µg/ml of CLP and 30 µg/ml MLT) and the solution filtered through Whatman filter paper No.41. This solution was use at stock solution 2 ml of aliquot solution was pipetted out and transferred to a 10 ml volumetric flask. Then the volume made up to the mark with methanol (100 µg/ml of CLP and 6 µg/ml of MLT). Then 5 ml was withdrawn from above working solution and transferred into 10 ml volumetric flask. Then the volume was made up to mark with methanol to get sample solution containing 50 µg/ml of CLP and 3 µg/ml of MLT respectively.

Estimation CLOMIPHENE CITRATE and MELATONIN.

1.At 324 nm (CLOMIPHENE CITRATE), $C_x = A_1 /0.00851$

2.At 225 nm (MELATONIN), $C_y = A_2 – 0.03010C_x /0.1521$

Where A_1, A_2 are absorbance of mixture at 324 nm and 225 nm.

C_x and C_y are concentration of CLOMIPHENE CITRATE and MELATONIN.

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III. RESULT AND DISCUSSION

➢ **FIRST ORDER DERIVATIVE METHOD**

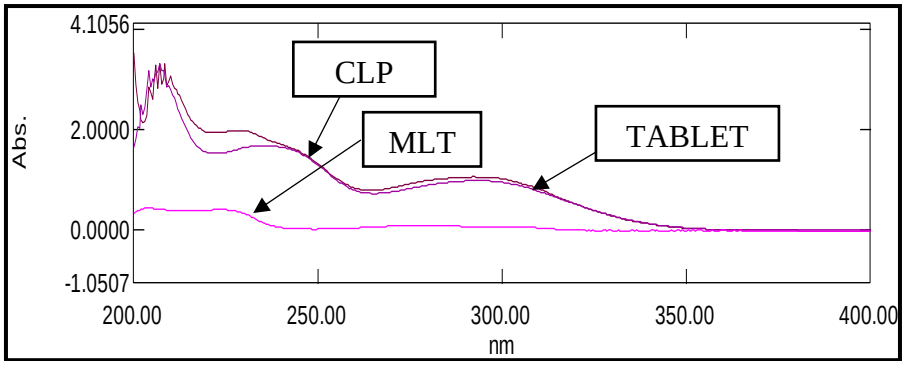

Fig. No.1 Overlain of CLP (50 µg/ml), MLT (3 µg/ml) and TABLET (50+3 µg/ml)

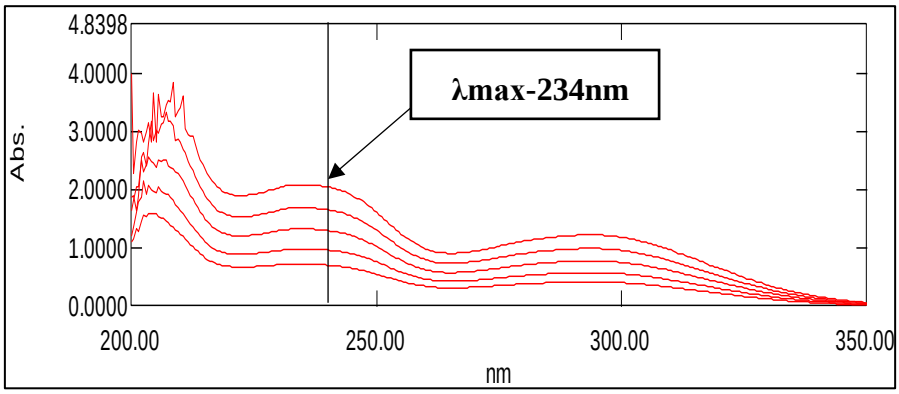

Fig. No.2 Zero Order Spectra of CLP (20-60 µg/ml)

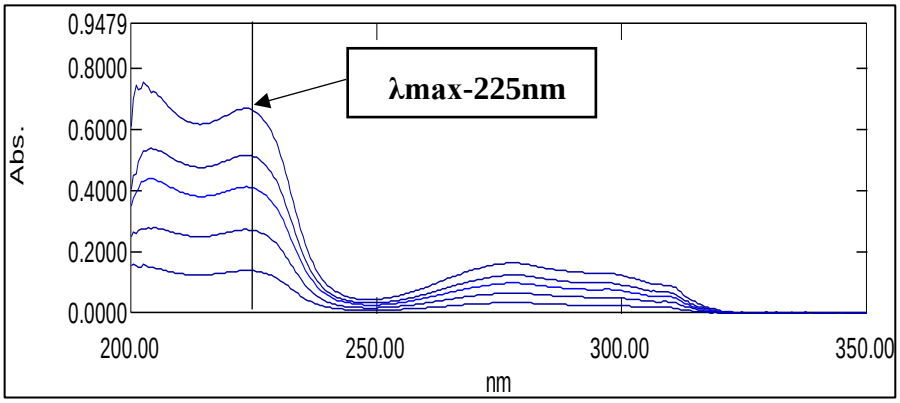

Fig. No.3 Zero Order Spectra of MLT (1-5 µg/ml)

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Selection of wavelength for estimation of CLP and MLT

To determine wavelength for estimation, standard spectra of CLP and MLT was scanned between 200-400 nm against methanol as blank. Zero Crossing Point were obtained at 249 nm and 234.5 nm for estimation of CLP and MLT respectively since adequate absorbance is produced at these wavelengths. Overlay first order spectra of CLP and MLT are presented in figure

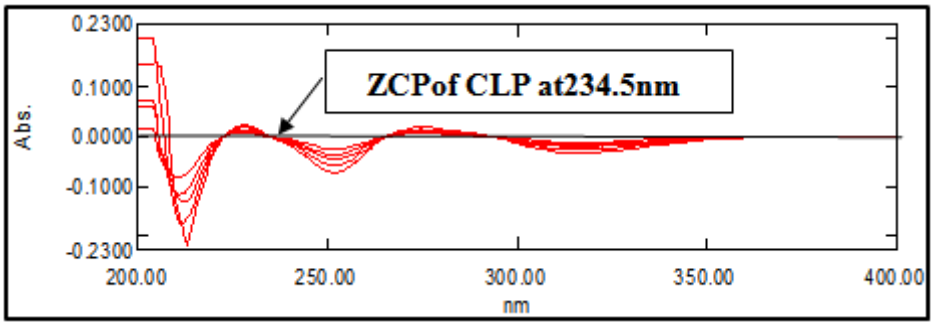

Fig.4 ZCP of CLP at 234.5 nm

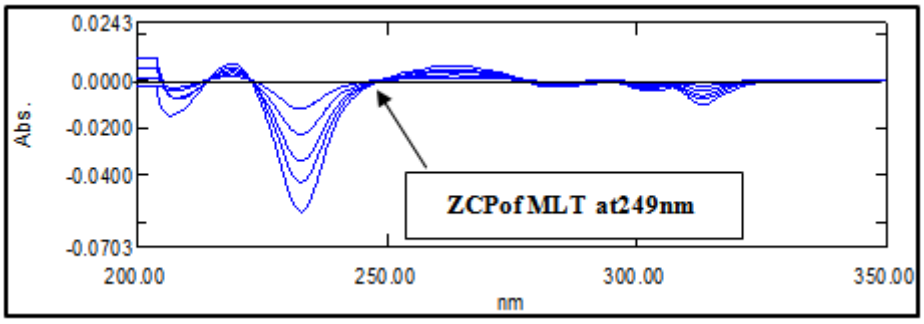

Fig.5 ZCP of MLT at 249 nm

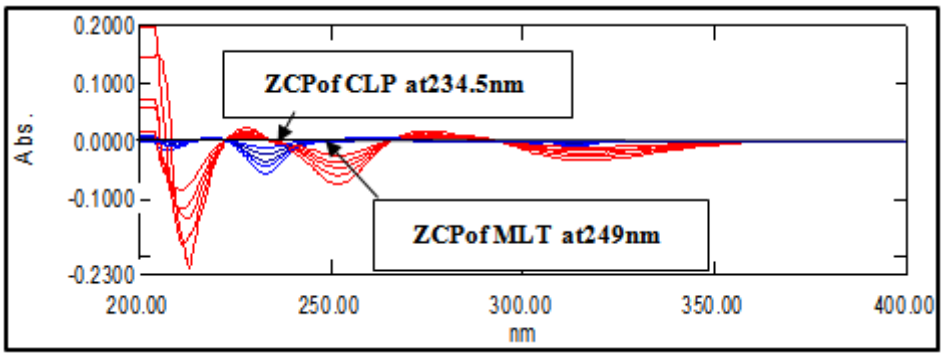

Fig.6 Overlain Spectra of CLP (20-60 µg/ml) and MLT (1-5) µg/ml)

VALIDATION OF FIRST ORDER DERIVATIVE METHOD

1. Linearity:

Table no. 1 Linearity of CLP at 249 nm and MLT at 234.5 nm

Sr. No.	Linearity of CLP at 249 nm (ZCP of MLT)			Linearity of MLT at 234.5 nm (ZCP of CLP)		
	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	% R.S.D.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	% R.S.D.
1.	20	0.0232 ± 0.000178	0.7684	1	0.0111 ± 0.000083	0.7523

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2.	30	0.0343 ± 0.000181	0.5290	2	0.0211 ± 0.000130	0.6156
3.	40	0.0423 ± 0.000177	0.4203	3	0.0322 ± 0.000130	0.4039
4.	50	0.0542 ± 0.000130	0.2402	4	0.0402 ± 0.000130	0.3236
5.	60	0.0672 ± 0.000114	0.1695	5	0.0512 ± 0.000122	0.2392

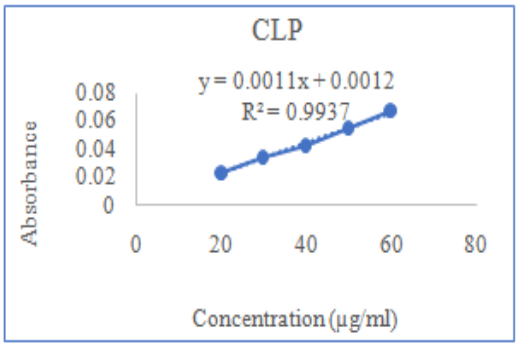

Fig.7 Calibration Curve of CLP at 249 nm

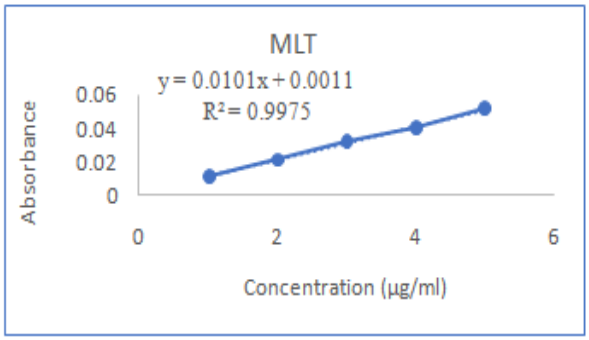

Fig.8 Calibration Curve of MLT at 234.5 nm

Table no.2 Regression line equation, Regression, coefficient and correlation coefficient for CLP and MLT

Sr. No.	Drugs	Regression line Equation	Regression coefficient(R^2)	Correlation coefficient(r)
1.	CLP	y =0.0011x + 0.0012	0.9937	0.9968
2.	MLT	y =0.0101x +0.0011	0.9975	0.9987

2. PRECISION

a) Repeatability (n=6):

Table no.3 Repeatability data for CLP at 249 nm (ZCP of MLT) and MLT at 234.5 nm (ZCP of CLP)

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
1.	CLP	50	0.0544 ± 0.000179	0.3288
2.	MLT	3	0.0324 ± 0.000187	0.5765

B) Intraday Precision (n=3):

Table no.4 Intraday Precision for CLP at 249 nm (ZCP of MLT) and MLT at 234.5 nm (ZCP of CLP)

Drugs	Intraday Precision			Interday Precision		
	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
CLP	40	0.0424 ± 0.000252	0.5930	40	0.0425 ± 0.000400	0.9411
	50	0.0542 ± 0.000252	0.4640	50	0.0544 ± 0.000351	0.6447
	60	0.0673 ± 0.000153	0.2267	60	0.0675 ± 0.000404	0.5984
MLT	2	0.0212 ± 0.000153	0.7182	2	0.0214± 0.000208	0.9697
	3	0.0325 ± 0.000200	0.6153	3	0.0324 ± 0.000252	0.7759
	4	0.0403 ± 0.000200	0.4962	4	0.0404 ± 0.000265	0.6548

3. Accuracy:

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Table no.5 Determination Accuracy of CLP and MLT

Drugs	Level	Amount of sample(µg/ml)	Amount of std. spiked(µg/ml)	Total amount (µg/ml)	amount of sample found(µg/ml)	% Recovery
CLP	0%	25	0	25	24.96	99.87
	80%	25	20	45	44.96	99.93
	100%	25	25	50	49.87	99.75
	120%	25	30	55	54.93	99.88
MLT	0%	1.5	0	1.5	1.49	99.66
	80%	1.5	1.2	2.7	2.69	99.86
	100%	1.5	1.5	3	2.99	99.77
	120%	1.5	1.8	3.3	3.29	99.90

3.1.3 ASSAY:

Table no.6 Analysis of marketed formulation

TABLET	Amount taken (mg/tab)		Amount Obtained (mg/tab) S.D. (n=5)		CLP %Purity ± S.D. (n=5)	MLT %Purity ± S.D. (n=5)
	CLP	MLT	CLP	MLT	CLP	MLT
	50	3	49.80 ±0.2170	2.98 ±0.0080	99.60 ±0.4340	99.48 ±0.2697

➢ **ABSORBANCE CORRECTION METHOD**

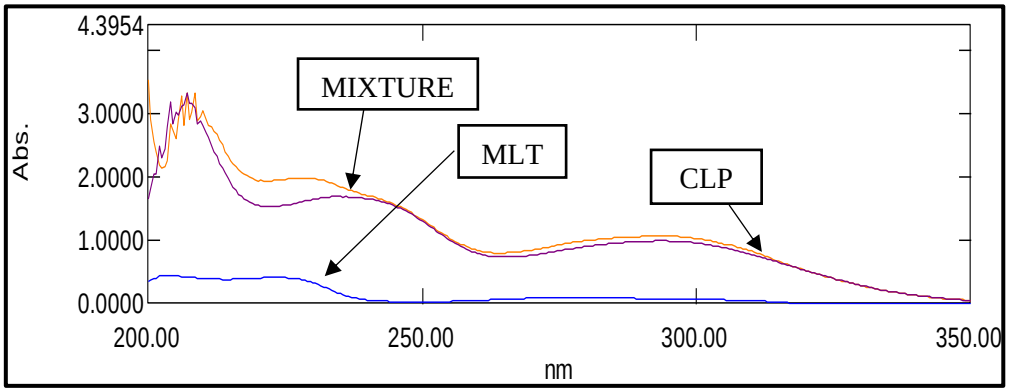

Fig. No.9 Overlain of CLP (50 µg/ml), MLT (3 µg/ml) and Mixture (50+3 µg/ml)

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SELECTION OF WAVELENGTH FOR ESTIMATION OF CLOMIPHENE CITRATE AND MELATONIN

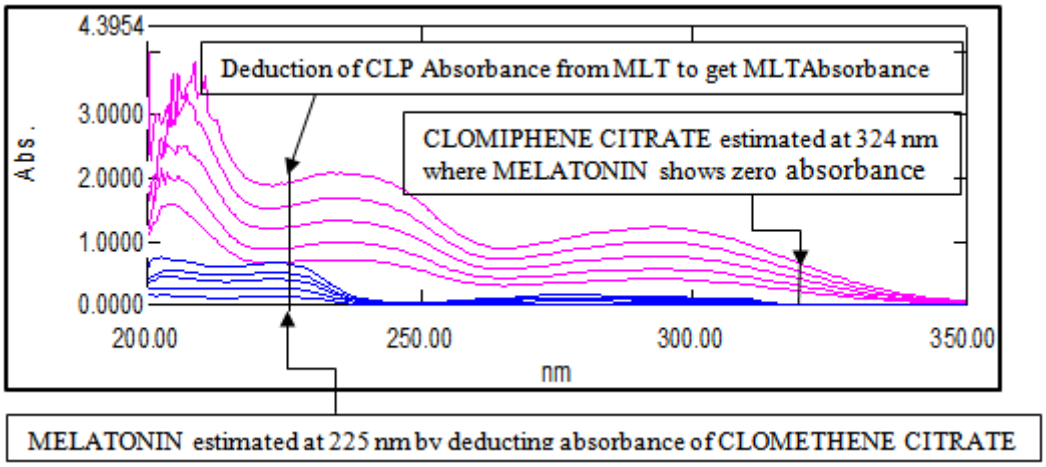

Fig. No.10 Selection of Wavelength for Estimation of Clomiphene Citrate and Melatonin

➢ **VALIDATION OF ABSORBANCE CORRECTION METHOD**

1.Linearity:

Table no.7Linearity data for CLP at 234 nm and MLT at 225 nm

	CLP at 234 nm			CLP at 225 nm		
Sr. No.	**Concentration (µg/ml)**	**Mean Abs. ± S.D. (n=5)**	**% R.S.D.**	**Concentration (µg/ml)**	**Mean Abs. ± S.D. (n=5)**	**% R.S.D.**
1.	20	0.1812 ± 0.00111	0.6122	1	0.4967 ± 0.00150	0.3020
2.	30	0.2428 ± 0.00109	0.4502	2	0.6308 ± 0.00133	0.2107
3.	40	0.3286 ± 0.00114	0.3459	3	0.8201 ± 0.00144	0.1759
4.	50	0.4271 ± 0.00103	0.2418	4	1.0372 ± 0.00116	0.1117
5.	60	0.5250 ± 0.00075	0.1434	5	1.2594 ± 0.00135	0.1072

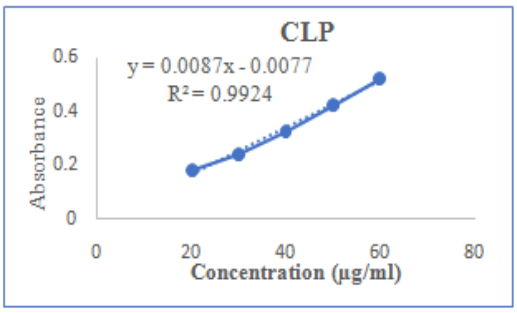

Fig. No.11 Calibration curve for CLP 234 nm

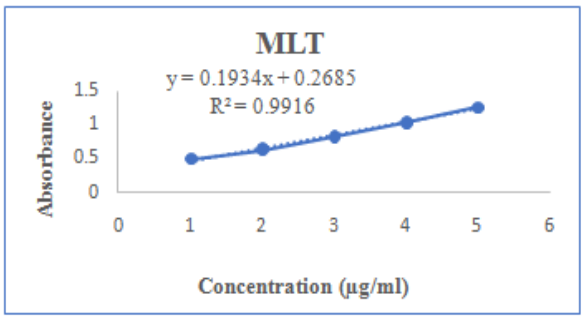

Fig. No.12 Calibration curve for MLT at 225 nm

Table no.8 Correlation Coefficient, Regression Coefficient and Regression Line Equation for CLP and MLT.

Sr.No.	Drugs	Regression line equation	Regression coefficient (R^2)	Correlation coefficient(r)
1.	CLP	y = 0.0087x - 0.0077	0.9924	0.9961
2.	MLT	y = 0.1934x + 0.2685	0.9916	0.9957

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1. Precision

a) Repeatability:

Table no.9 The data for repeatability for CLP at 324 nm and MLT at 225 nm is shown

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
1.	CLP	50	0.4273 ± 0.00130	0.3053
2.	MLT	3	0.8206 ± 0.001661	0.2023

b) Intraday Precision and Interday precision

Table no.10 The data for intraday precision and Interday precision for CLP at 324 nm and MLT at 225 nm

Drugs	intraday precision			Interday precision		
	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=3)	%R.S.D.
CLP	40	0.3292 ± 0.00172	0.5236	40	0.3296 ± 0.00211	0.6409
	50	0.4275 ± 0.00175	0.4093	50	0.4280 ± 0.00231	0.5389
	60	0.5256 ± 0.00169	0.3219	60	0.5261 ± 0.00219	0.4168
MLT	2	0.6321 ± 0.00296	0.4677	2	0.6326 ± 0.00325	0.5139
	3	0.8209 ± 0.00252	0.3074	3	0.8216 ± 0.00332	0.4038
	4	1.0380 ± 0.00212	0.2044	4	1.0392 ± 0.00326	0.3137

d) Accuracy:

Table no.11 The data of accuracy for CLP at 324 nm and MLT at 225 nm

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
CLP	0%	25	0	25	24.96	99.87
	80%	25	20	45	44.83	99.63
	100%	25	25	50	49.98	99.96
	120%	25	30	55	54.58	99.24
MLT	0%	1.5	0	1.5	1.49	99.89
	80%	1.5	1.2	2.7	2.69	99.86
	100%	1.5	1.5	3	2.99	99.89
	120%	1.5	1.8	3.3	3.29	99.81

ASSAY:

Table no.12 Analysis of marketed formulation

TABLET	Amount taken (mg/tab)		Amount Obtained (mg/tab)		CLP %Purity ± S.D. (n=5)	MLT %Purity ± S.D. (n=5)
	CLP	MLT	CLP	MLT	CLP	MLT
	50	3	49.97 ± 0.0065	2.98 ± 0.0101	99.94 ± 0.0117	99.61 ± 0.3366

IV. CONCLUSION

A. FIRST ORDER DERIVATIVE METHOD

Based on the results, obtained from the analysis of CLP and MLT in their Tablet dosage form using First Order Derivative Method, it can be concluded that the method has linearity in the range of 20-60 µg/ml for CLP and 1-5 µg/ml for MLT. The regression coefficient (R^2) was found to be 0.9937 and 0.9975 for CLP and MLT and correlation coefficient (r) was found to be 0.9968 and 0.9987 for CLP and MLT at 249 nm (ZCP of MLT) and 234.5 nm (ZCP of CLP) respectively. Limit of detection for CLP and MLT were found to be 0.6906 µg/ml and 0.0278 µg/ml and Limit of quantification for CLP and MLT were found to be 2.0928 µg/ml and 0.0845 µg/ml respectively. The % assay was found to be 99.60 % and 99.48 % for CLP and MLT respectively. Further % R.S.D. was found to be less than 2% for repeatability, intraday and interday study.

B. ABSORBANCE CORRECTION METHOD

Based on the results, obtained from the analysis of CLP and MLT in their Tablet Dosage

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Form using Absorbance Correction Method, it can be concluded that the method has linearity in the range 20-60 µg/ml for CLP and 1-5 µg/ml for MLT .The regression coefficient (R^2) were found to be 0.9924 and 0.9978 for MLT and correlation coefficient was found to be 0.9961 and 0.9988 for CLP and ML at 324 nm and 225 nm respectively. Limit of detection for CLP and MLT were found to be 0.5182 µg/ml and 0.0301 µg/ml and Limit of quantitation for CLP and MLT were found 1.5705 µg/ml and 0.0913 µg/ml respectively. The % assay was found to be 99.94 % and 99.61 % for CLP and MLT respectively. Further % R.S.D was found to be less than 2% for repeatability, intraday and Interday precision study.

REFFERENCES:

[1]. Introduction of Drug, "Combination of Clomiphene Citrate and Melatonin", February 2020, https://www.1mg.com/generics/clomiphene-melatonin-404253

[2]. Drug Profile, "Clomiphene Citrate", October 2023,https://go.drugbank.com/salts/DBSALT000490

[3]. Drug Profile, "Melatonin", October 2023, https://go.drugbank.com/categories/DBCAT001822

[4]. A Patel, B Shah "Development and validation of Area Under Curve method for simultaneous Estimation of Thiocolchicoside and Lornoxicam in Tablet Dosage form" J Pharm Sci Bio Res, 2014, 4 (6), 383-387.

[5]. Chauhan A, hartiMittu B, Chauhan P. "Analytical Method Development and Validation: A Concise Review." J Anal Bioanal Tech. 2015, 1(6), 233.

[6]. Patel Smit, Raulji Aniket, Patel Diya, Panchal Diya, Prof. MitaliDalwadi, Dr. Umesh Upadhyay. "A Review on UV Visible Spectroscopy." Int. j. pharm. res. appl. 2022, 7(5), 1144-1151.

[7]. Patel AP, Kadikar HK, Shah RR, Shukla MH "Analytical method development and validation of First order derivative spectroscopic method for simultaneous estimation of Cinnarizine and Dimenhydrinate in combined dosage form" Pharma Science Monitor An international journal of pharmaceutical sciences, 2012, 1 (1), 2493-2505.

[8]. Soni Abhishek, Chaudhary Amit, Singla Shivali, Goyal Sachin, "Development and Validation of The UV Spectrophotometric Method of Clomiphene Citrate in Simulated Vaginal Fluid and Stress Degradation Studies." IJPSR. 2019, 8(10), 2277- 8616.

[9]. Joshi K, Shah N, Dumasiya M, Patel A "Development and Validation of Spectrophotometric Method for Estimation of Lurasidone Hydrochloride: A novel antipsychotic drug in bulk and pharmaceutical dosage form" Pharma Science Monitor An international journal of pharmaceutical sciences, 2012, 3(4), 2643-2653.

[10]. Vaghasiya Nirav, and Antala Hiren, "Development and validation of first order derivative spectrophotometric method for estimation of clomiphene citrate in bulk drug and formulation." J. Pharm. Anal. 2013, 2(1), 107- 114.

[11]. Shukla M, Patel A, Patel M, Patel P, Shah R "Development and Validation of First order derivative spectroscopic method for the Estimation of Olopetadine Hydrochloride and Ambroxol Hydrochloride in their synthetic mixture" Pharm Sci Monitor, 2015, 6(1), 119-131.

[12]. Dr. Alisha Patel, Heli Desai, Ankita Patel, "Derivatization of Neomycin Sulphate and Area Under Curve Method for Estimation of Neomycin Sulphate and Clobetasol Propionate in Cream" Journal of advanced scientific research, 2022, 13 (9), 88-93.

[13]. Vaddempudi, Venkatakiran, et al., "Development and validation of zero order spectrophotometric method for estimation of clomiphene citrate in bulk and tablet dosage form." Int. J. Pharmtech Res. 2013, 5(1), 27-30.

[14]. Yukti Patel, Dr. Alisha Patel, "Absorbance Ratio Spectroscopic Method Developmentand Validation for Simultaneous Estimation of Dapagliflozin PropanediolMonohydrate and Linagliptin" International Journal of Pharmaceutical research andApplications, 2023, 8 (4), 29-40.

[15]. Hassan, Wafaa S, and Mervat M. Hosny, "Spectrophotometric and conductometric determination of Clomiphene citrate and Nefazodone HCL." E- J. Chem. 2008, 5(S2), 1069-1080.

[16]. Tank PK, Shah RR, Shukla MH, Nayak PP, Patel AP "Development and Validation ofFirst order derivative spectroscopic method for the Estimation of Thiocolchicosideand Dexketoprofen Trometamol in Pharmaceutical dosage form" Pharm Sci Monitor,2012, 3(4), 2613-2622.