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Development And Validation Of Zero Order Spectrophotometric Method For Estimation Of Clomiphene Citrate In Bulk And Tablet Dosage Form

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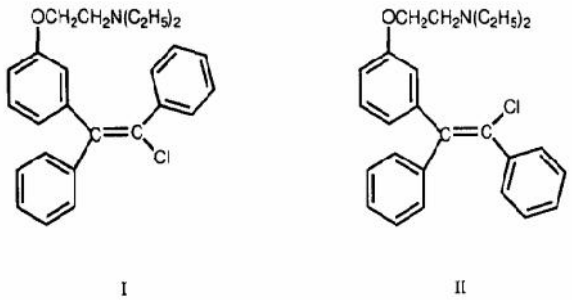
Abstract: The present study describes simple, accurate, precise and cost effective UV Spectrophotometric method for the estimation of Clomiphene citrate in bulk and tablet dosage form. The solvent used was Acetonitrile and the absorption maxima for Clomiphene citrate was found to be 234 nm and 290nm. A linear response was observed in the range of 4-20µg/mL with a correlation coefficient of 0.999 for Clomiphene citrate .The method was then validated for different parameters like accuracy, precision, sensitivity and linearity as per ICH Q2 guidelines. This method can be used for the estimation of Clomiphene citrate in quality control of formulation without interference of excepients. The method was successfully applied to estimation of Clomiphene citrate in tablet formulations.
Keywords: UV-Spectrophotometry, Clomiphene citrate , Method development.

INTRODUCTION [1-2]

Clomiphene is a non steroidal compound which has estrogenic and anti-estrogenic effect. Clomiphenecitrate is a mixture of Z isomer (zuclomiphene) and the E isomer (enclomiphene) and contain not less than 30%and not more than 50% of the Z isomer. Chemical Name of Clomiphene citrate is Ethanamine,2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-*N*,*N*-diethyl-,2-hydroxy-1,2,3-propanetricarboxylate. Clomiphene citrate is available as tablet dosage form (siphene) and is used as anti oestrogenic. Clomiphene citrate is used for induction of ovulation. Z-Isomer of clomiphene citrate is have the therapeutic activity



I II

Fig. 1. Structures of enclomiphene (I) and zuclomiphene (II).

2.EXPERIMENTAL[3-8]

Instrument The instrument used for the study was a UV-Visible spectrophotometer (SHIMADZU UV-1800 240V) having two matched quartz cells with 1cm path length. The solvent used was Acetonitrile (AR grade).

Reagents and Chemicals
Pure samples of Clomiphene citrate was obtained from Micro Labs Limited, Hosur, India. The commercial pharmaceutical preparation Siphene containing 100mg Clomiphene citrate (Marketed by Ltd) was procured from local pharmacy. The solvent used was Acetonitrile AR grade) from SD fine-chem Limited, Mumbai, India.

Solubility test
Solubility test for Clomiphene citrate was performed by using various solventsi.e., methanol, water, Acetonitrile. The drug was freely soluble in Acetonitrile. Hence Acetonitrile was selected as a solvent for the proposed method. Working standard solutions of the drug was scanned in the UV range of 200 to 400nm, using acetonitrile as blank. acetonitrile showed good absorption at 234 and 290 nm . 290 nm was selected for determination due to maximum absorption of therapeutic active Z-Isomer at 290 nm.

Preparation of working Standard solution of Clomiphene citrate
Accurately weighed 25mg of Clomiphene citrate was transferred in to clean and dry separate 25mL volumetric flask and dissolved with sufficient volume of Acetonitrile. The volume was made up to 25mL with Acetonitrile to get concentration of 1000µg/mL. From that 5mL of stock solution was diluted in 50 mL volumetric flask with Acetonitrile to get a concentration 100µg/mL.
From above solution,0.4,0.8,1.2,1.6,2.0 mL of the stock solutions was further diluted to a five 10mL volumetric flasks individually with acetonitrile to get a concentration 4,8,12,16,20µg/mL.

Analysis of marketed formulations
Twenty tablets were weighed and finely powdered. The powder equivalent to 100 mg of Clomiphene citrate was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 50 ml of the Acetonitrile and sonicated for 30 min. This solution was carefully filtered through Whatmann filter paper (No. 41) and the final solution was made with Acetonitrile to get the solution of 1000 mcg / ml. From this solution, 10ml was taken in 100 ml standard volumetric flask and diluted to 100 ml with Acetonitrile to give a solution of 100 mcg / ml.

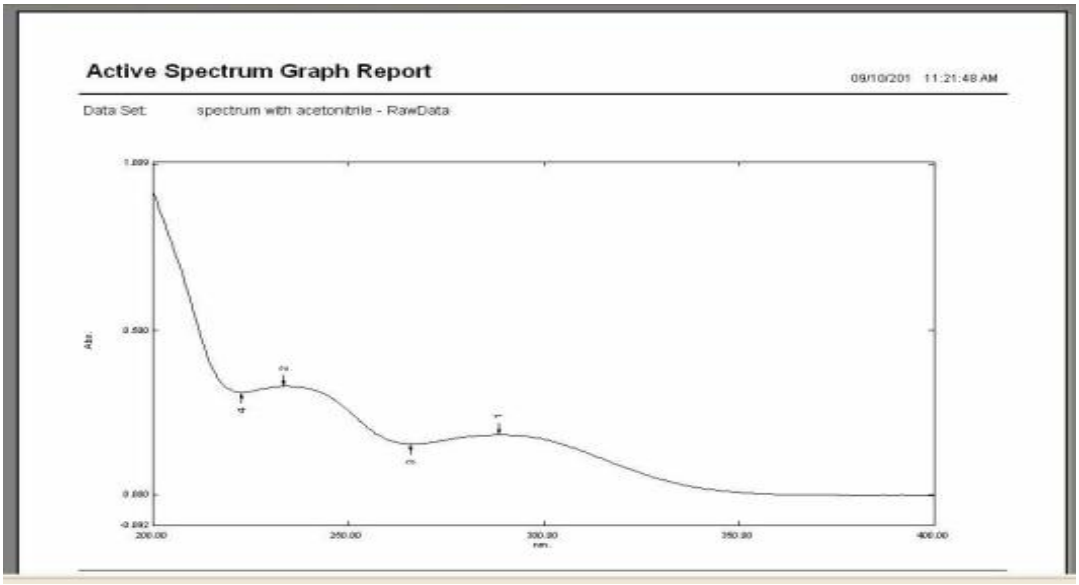


Fig No.2: UV-Spectra of Clomiphene

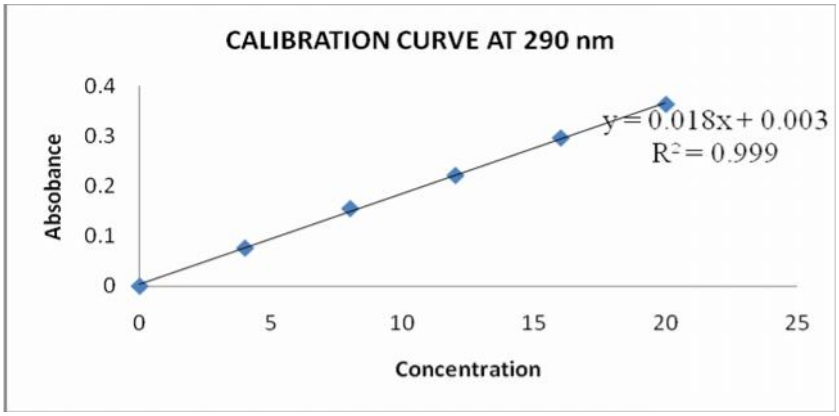


Fig No.3: Calibration curve for CC at 290 nm

Table 1: Determination of Accuracy results of Clomiphene citrate by Zero order Spectroscopy

Tablet	Amount of sample (mcg / ml)	Amount of drug added (mcg / ml)	Amount Recovered (mcg / ml)	% Recovery ± SD**
Siphene	12	6	18.05	100.9 ± 0.55
	12	12	24.06	100.52 ± 0.33
	12	18	30.09	100.50 ± 0.24

**Average of Six determinations

Table 2: Determination of Precision results of Clomiphene citrate at 290 nm by Zero order Spectroscopy

Conc. (mcg / ml)	Intra-day Absorbance Mean ± SD**	% CV	Inter-day Absorbance Mean ± SD**	% CV
4	0.077 ± 0.000983	1.28	0.076 ± 0.000753	0.99
8	0.154 ± 0.001049	0.68	0.155 ± 0.001722	1.11
12	0.222 ± 0.00216	0.97	0.222 ± 0.001835	0.83
16	0.293 ± 0.001751	0.60	0.293 ± 0.001414	0.48
20	0.360 ± 0.002639	0.73	0.359 ± 0.002422	0.67

**Average of Six determination

RESULTS AND DISCUSSION

The method was validated according to the ICH guidelines with respect to linearity, accuracy, precision and ruggedness.

The calibration plot for the method was linear over the concentration range of 4-20µg/mL for Clomiphene citrate. The determination of coefficients (r^2) was 0.999.. The method was found to be precise and as the %RSD values for intraday and inter day were found to be less than ±2%. % recovery (98-99%) was found to be good at each added concentration, indicating that method was accurate. The LOD and LOQ were found to be 0.19µg/mL and 0.56µg/mL The sensitivity of the method was found to be 0.054299µg/cm^3/AU. The results of assay showed that the amount of drug was in good agreement with the label claim of formulation as indicated by % assay 100.24.

Table 3: Analysis of tablet formulation

Parameter	UV method
λ_{max} (nm)	290
Beer's law limits (mcg / ml)	4-20
Molar extinction coefficient ($L.mol^{-1} cm^{-1}$)	$0.01842X10^4$
Sandell's sensitivity (mcg / cm^2-0.001 absorbance units)	0.054299
Regression equation (Y*)	Y = 0.019 C + 0.003
Slope (b)	0.019
Intercept (a)	0.003
Correlation coefficient (r^2)	0.999
Intraday Precision (% RSD**) Interday Precision (% RSD**)	0.97 0.83
Limit of detection (mcg / ml)	0.19
Limit of quantitation (mcg / ml)	0.56

**Average of Six determination

Table.No:4 Optimum conditions, Optical characteristics and Statistical data of the Regression equation in Zero order Spectroscopy

Tablet (Siphene)	Label Claim (mg)	Analyst I		Analyst II	
		Amt found (mg)	(%)Recovery ± SD**	Amt found (mg)	(%)Recovery ± SD**
Sample I	100	100.24	100.24± 0.72	100.36	100.36 ± 0.66
Sample 1I	100	100.39	100.39 ± 0.67	100.22	100.22 ± 0.67

CONCLUSION

A simple, accurate, fast and precise UV- Zero order method has been developed for the estimation of Clomiphene citrate. The proposed method is successfully applied for estimation of drug in bulk and tablet dosage forms. The method can be used for the routine quality control analysis of Clomiphene citrate in combined dosage form.

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