Research Article **CODEX: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CLOMIPHENE CITRATE IN BULK DRUG AND FORMULATION

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ABSTRACT

A simple, accurate, precise and sensitive First order derivative Spectrophotometric method was developed for the estimation of Clomiphene Citrate in bulk and pharmaceutical dosage forms. The estimation of Clomiphene Citrate was carried out at maximum absorbance of 293 nm. The method was found to be linear and obeys Beer's law in the concentration range of 10-50 mcg / ml. The developed method was validated according to ICH guidelines and was found to be accurate and precise. Thus the proposed method can be successfully applied for the estimation of Clomiphene Citrate in bulk and pharmaceutical dosage forms.

KEYWORDS

Clomiphene Citrate, Validation, ICH guidelines and First order derivative spectroscopy.

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INTRODUCTION[1, 3]

Anti-estrogens are crucial for safe and effective anabolic steroid usage. Clomiphene citrate 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. Clomiphene is primarily used for the treatment of an ovulatory infertility. It has also been used in the treatment of male infertility. Very few methods were reported for determination of Clomiphene citrate by U.V Spectroscopy. So, in the present

study, a simple, precise and accurate U.V Spectroscopy method was developed (Figure No.1). Clomiphene Citrate is a white or almost white powder. It is freely soluble in Methanol and Insoluble in Water. The drug is officially listed in monograph of IP 2007. Several analytical methods that have been reported for the estimation of Clomiphene Citrate in biological fluids or pharmaceutical formulation include Conductometry and UV-Visible Spectrophotometry. The objective of the work was to develop simple, accurate, precise and economic First order derivative Spectroscopic method to estimate the Clomiphene Citrate in bulk and pharmaceutical dosage forms. The method is simple, reproducible and statistically valid. UV-VIS Spectrophotometer Shimadzu UV-1800 with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement. Methanol was of analytical grade.

100 mg of standard Clomiphene Citrate drug was weighed, transferred to a 100 ml volumetric flask and dissolved in Methanol. The flask was shaken and volume was made up to the mark with Methanol to give a solution containing 1000 µg / ml. From this stock solution, 10 ml of solution was pippeted out and placed into 100ml volumetric flask. The volume was made up to mark with Methanol to give a solution containing 100 µg / ml. From the standard stock solution of Clomiphene Citrate, appropriate aliquots were pippeted out in to 10 ml volumetric flasks and dilutions were made with Methanol to obtain working standard solutions of concentrations from 10 to 50 mg / ml. Absorbance for these solutions were measured at 293 nm. For the standard solution analytical concentration range was found to be 10-50 µg / ml. Appropriate volume of aliquots from standard Clomiphene Citrate stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with Methanol to obtain concentrations of 10, 20, 30, 40, and 50 µg / ml. Absorbance spectra of each solution against Methanol as blank were measured at 293 nm. The regression equation and correlation coefficient was determined. Twenty Tablets of two brands were taken, powder equivalent to 100 mg of Clomiphene Citrate was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the Methanol and sonicated for 5 min. The flask was shaken and volume was made up to the mark with Methanol to give a solution of 1000 µg / ml. The above solution was centrifuged at 2000 rpm for 10 minutes and carefully filtered through Whatmann filter paper (No.41). From this solution, 10ml was taken and diluted to 100 ml with Methanol to give a solution of 100 µg / ml and used for the estimation of Clomiphene Citrate. To examine the absence of either positive or negative interference of excipients used in formulation, recovery studies were carried out. Accuracy was determined by recovery studies. The recovery studies were carried out by adding the known amount of Standard Clomiphene Citrate drug to the sample solution of the capsules. Precision for assay were determined by repeatability, interday, intraday precision for drug (each in three replicate). Ruggedness studies were carried out by changing the analysts.

MATERIAL AND METHODS

MATERIALS

Pure sample of Clomiphene citrate was obtained from Palam Pharma Ltd, Ahmedabad, Gujarat, India. The commercial pharmaceutical preparation Siphene containing 25mg of Clomiphene citrate (Marketed by Serum Institute of India Ltd) were procured from local pharmacy. Methanol was obtained from Merck Mumbai, India. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

METHODS

Standard and sample preparation

The standard stock solution of Clomiphene citrate of 1000 mcg/mL was prepared by dissolving working standards in small proportion of Methanol upto sufficient volume. Standard calibration solution of having concentration in the range of 10-50 mcg/mL respectively were prepared by diluting stock solution with Methanol.

Analysis of dosage form

Twenty tablets were weighed their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to one tablet content was transferred into a 100ml volumetric flask containing 10 ml of Methanol, mechanically shaken for 10 min, ultrasonicated for 5 min, and then diluted to volume with Methanol.

Experimental Section[4-10]

Calibration curves citrate of Clomiphene citrate

The calibration curves were plotted over a concentration range of 10-50 µg/ml for Clomiphene Citrate Table No.1 and Figure No.2. Accurately measured standard solutions of Clomiphene Citrate (0.1, 0.2, 0.3, 0.4, 0.5) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First-derivative absorbance (D1) was measured at 293.00 nm for Clomiphene Citrate (Figure No.3). The calibration curves were constructed by plotting absorbance versus concentrations and the regression equations were calculated.

Linearity of First Order Derivative Spectra of Clomiphene Citrate

Zero Crossing Point was Found to be 293.00 nm for First order derivative spectra of Clomiphene Citrate.

Accuracy

Procedure

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Clomiphene citrate API was added in placebo to attain 50%, 100% and 150% of sample concentration. Amount as shown above was weighed and transferred into 100 ml volumetric flask and 50 ml of diluent was added. The volumetric flask was sonicated to disperse tablets completely for 30 minutes with intermittent shaking. The solution was cooled to the room temperature and made up to volume with diluent. 5 ml of this solution was diluted to 50 ml with diluents. The solution was filtered through 0.45 µ Millipore PVDF filter; filtrate was collected after discarding first few ml.

Each sample was prepared in triplicate at each level and injected. The chromatograms were recorded and from the peak area of drug, % recovery was calculated from regression equation of the calibration curve as shown in Table No.2.

Precision

Procedure

Method precision for assay was established by determining the assay of six sample preparations under same conditions. Six replicates of sample were prepared at sample concentration by one analyst and analyzed on same day (Table No.3).

Limit of Detection and Limit of Quantification[4]

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times SD/S$

$LOQ = 10 \times SD/S$

Where SD = the standard deviation of the response

S = Slope of calibration curve.

The value of LOD and LOQ are shown in Table No.6.

RESULTS AND DISCUSSION

A variety of analytical method development and validation of Clomiphene citrate by U.V method. The suitability of first order derivative spectroscopy was decided on the basis of selectivity and sensitivity of the assay, and separation among isomers during method development and validation. The maximum absorption wavelength of the reference drug was found to be 233 and 293 nm. When observed from the UV absorption spectra Figure No.3. 293 nm was selected as detection wavelength for analysis due to maximum absorbance of therapeutic Z-Isomer at 293 nm. Shimazdu 1800 U.V Spectrophotometer was used during analysis.

Method validation

The calibration plot for the method was linear over the concentration range of 10-50 mcg/mL. The determination of coefficients (*r2*) was 0.999. Values of the method Accuracy was calculated by recovery studies at three levels and found to be 98.47%. For precision % RSD of was within 2.0% thus confirm good precision of the analytical method development.

Table No.1: Linearity and Range

S.No	Concentration (µg/ml)	Absorbance
1	10	0.262
2	20	0.419
3	30	0.596
4	40	0.793
5	50	0.971

Table No.2: Accuracy

S.No	Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean % Recovery*
1	Clomiphene Citrate	I	20	50 %	98.00 %
2	Clomiphene Citrate	II	20	100 %	97.78%
3	Clomiphene Citrate	III	20	150 %	99.63%

***** Average of Six Determination

Table No.3: Precision

S.No	Absorbance at 293.0 nm (ZCP of Clomiphene Citrate)
1	0.419
2	0.418
3	0.421
4	0.417
5	0.420
6	0.419
Mean	0.419
S.D.	0.001414
% RSD	0.3374

Table No.4: Analysis of Clomiphene Citrate in Pharmaceutical Formulation

S.No	Concentration(µg/mL)	Absorbance	Amount Found(µg/mL)	Amount Found%
1	20	0.415	19.81	99.04
2	20	0.417	19.90	99.52
3	20	0.419	20.00	100.00
4	20	0.416	19.85	99.28
5	20	0.418	19.95	99.76
6	20	0.420	20.04	100.23
	Mean	0.418	19.93	99.64
	S.D	0.001871	0.08826	0.4463
	% RSD	0.4476	0.4428	0.4479

Table No.5: Analysis of Clomiphene Citrate by Proposed method

S.No	Concentration(µg/mL)	Absorbance	Amount Found(µg/mL)	Amount Found%
1	20	0.419	20.00	100.00
2	20	0.417	19.90	99.52
3	20	0.420	20.04	100.23
4	20	0.418	19.95	99.76
5	20	0.421	20.09	100.47
6	20	0.419	20.00	100.00
	Mean	0.419	19.99	99.99
	S.D	0.001414	0.06653	0.3351
	%RSD	0.3374	0.332	0.335

Table No.6: Characteristic parameters of Clomiphene citrate

S.No	Parameters	First-derivative Spectrophotometry
		Clomiphene Citrate at 293.0 nm
1	Concentration range (µg/ml)	10-50 µg/mL
2	Slope	0.017
3	Intercept	0.072
4	Correlation coefficient	0.999
5	LOD (µg/ml)	0.3631
6	LOQ (µg/ml)	1.1005
7	Recovery (Accuracy, n = 6), %	98.47%
8	Repeatability (RSD, n = 6), %	0.3374
9	Precision (RSD), %	0.3374

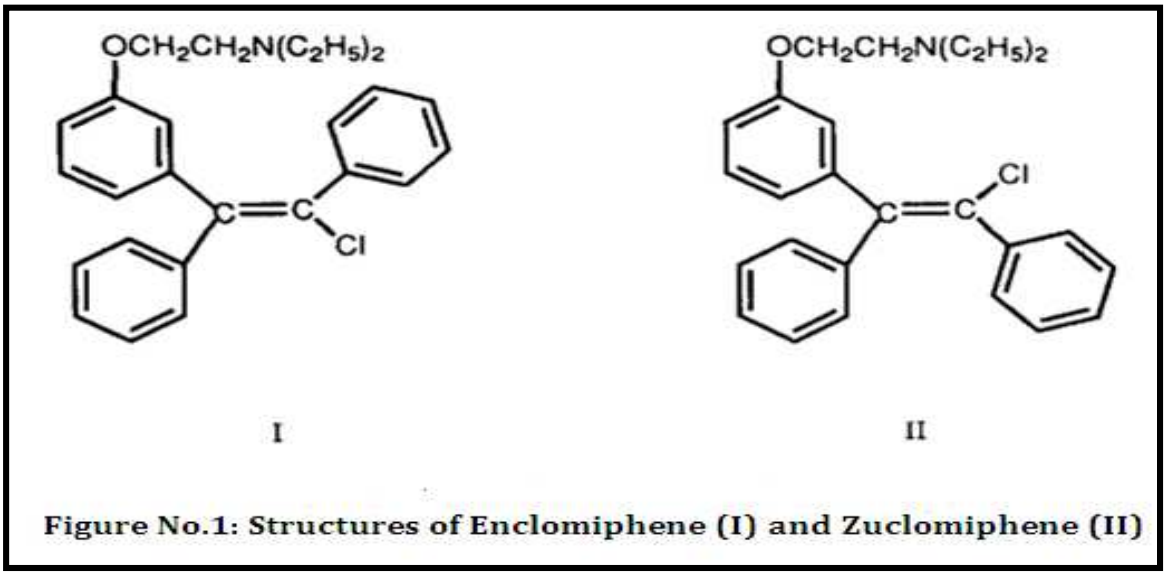

Figure No.1: Structures of Enclomiphene (I) and Zuclomiphene (II)

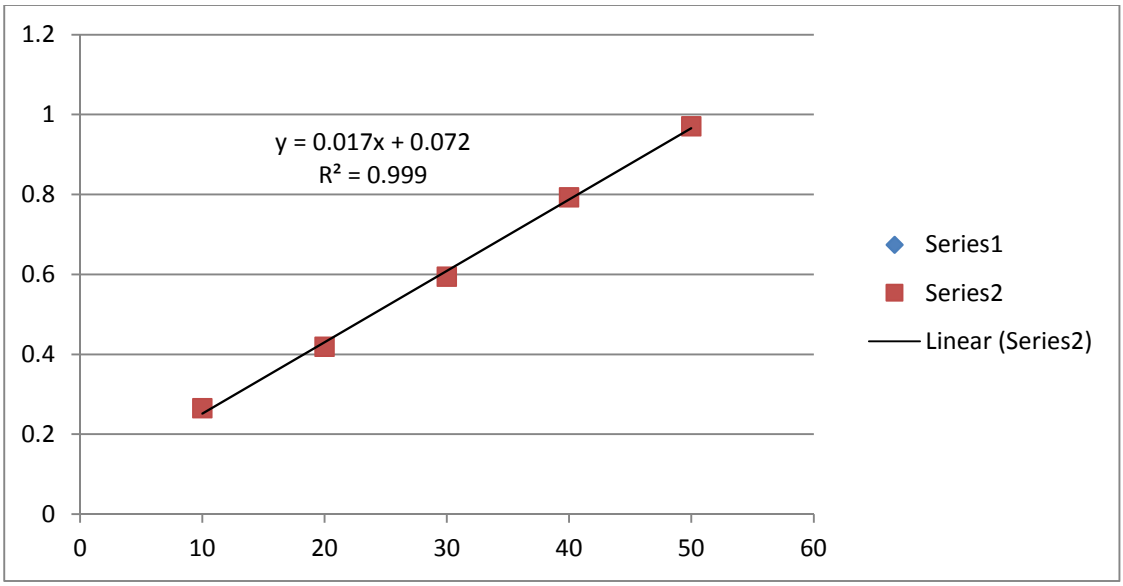

Figure No.2: Calibration curve citrate of Clomiphene citrate

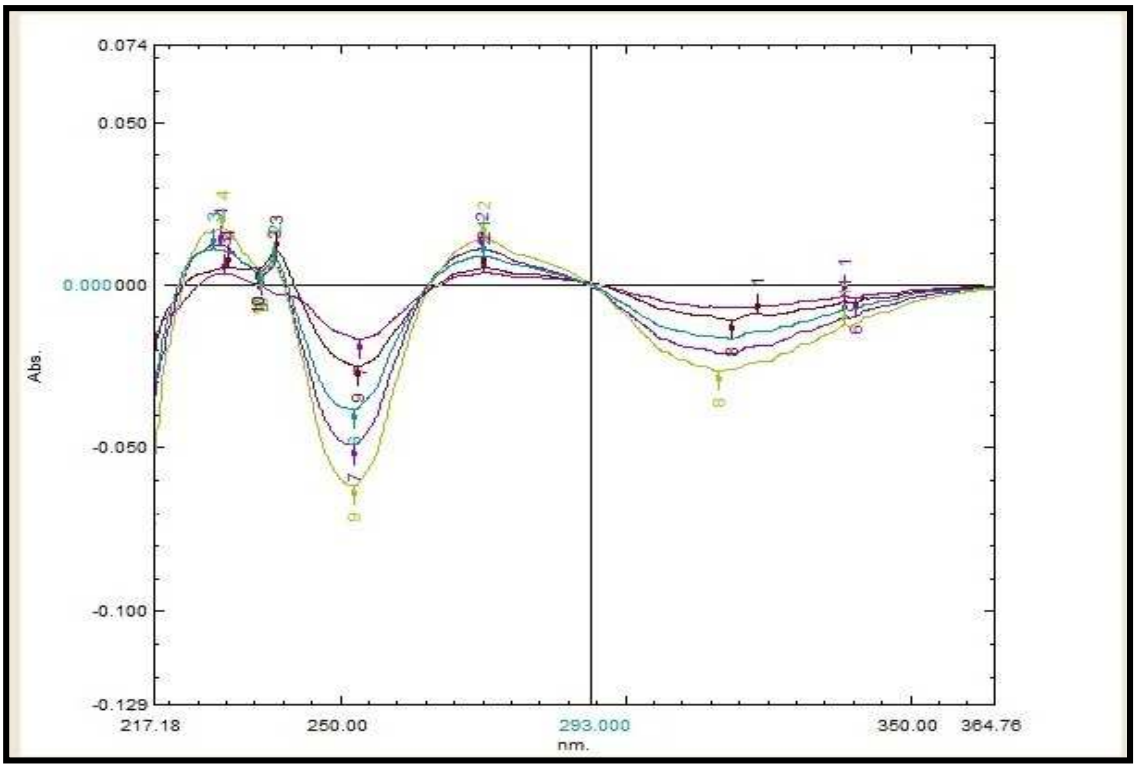

Figure No.3: ZCP of Clomiphene Citrate

CONCLUSION

The developed U.V technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Clomiphene citrate in bulk and pharmaceutical formulation without any interference from the excipients. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. Thus this method suitable for quantification of Clomiphene citrate in bulk drugs and in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The author thanks Palam Pharmaceuticals Ltd. Ahmedabad, Gujarat, India for providing the gift sample of Clomiphene citrate. I thank to the Noble college management for providing the facilities and chemical. And Sincere thanks to Mr. Hiren Antala for his Valuable Help.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Nirav Vaghasiya. *et al*. Development and validation of first order derivative spectrophotometric method for estimation of clomiphene citrate in bulk drug and formulation, *International Journal of Research in Pharmaceutical and Nano Sciences*, 2(1), 2013, 107-114.