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UV SPECTROPHOTOMETRIC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CLOMIPHENE CITRATE IN METHANOL

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Keywords:

Clomiphene citrate, Methanol, Method development

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ABSTRACT: The present study describes simple, accurate, precise and cost effective UV-spectroscopic method for the estimation of clomiphene citrate in methanol as per ICH guidelines. The absorption maxima for clomiphene citrate in methanol was found to be 235nm and 294 nm. The drug follows linearity (linearity 1, linearity 2 & linearity 3) in the concentration range 3-40 µg/ml at 294 nm and 3-24 µg/ml at 235 nm with a correlation coefficient value of 0.9991, 0.9989, 0.9988 and 0.999, 0.9994, 0.9994 respectively. The accuracy of the method was checked by recovery experiment performed at three levels *i.e.*, 80%, 100%, 120%. The percent recovery was found to be in the range of 90-120%. The low values of % RSD are indicative of accuracy and reproducibility of method. The % RSD <2 indicates that method is precise. The ruggedness of method was studied with the help of different analysts. No significant degradation of clomiphene citrate was showed in force degradation studies like heat-induced degradation the spectra for acid degradation, acid/base hydrolysis, oxidation and photo degradation. The above method was a rapid tool for estimation of clomiphene citrate in formulations.

INTRODUCTION: Clomiphene citrate is a non steroidal compound. Clomiphene citrate has been used for induction of ovulation since 1962. It is the treatment of first choice in women with ovulatory disorders who are normally oestrogenized, *i.e.* predominantly those with polycystic ovaries (PCO). Clomiphene citrate has both oestrogenic and anti-oestrogenic properties. Acting as an anti-oestrogen, clomiphene citrate is thought to displace endogenous oestrogen from hypothalamic and pituitary oestrogen receptor sites [1].

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Chemical Name of Clomiphene Citrate: [2-6]

- 2-Cp-(2-chloro-1, 2 diphenylvinyl) phenoxy] triethylamine citrate.
- Ethanamine, 2- [4- (2- chloro- 1, 2-diphenyl-ethenyl)-phenoxyl-N, N-diethyl-, 2-hydroxy-1,2,3-propanetricarboxylate.
- 2, 4, 2-chioro-1, 2-diphenylethenyl)-phenoxy]-N,N-diethylethanamine 2-hydroxy -1,2,3 propanetricarboxyIate.

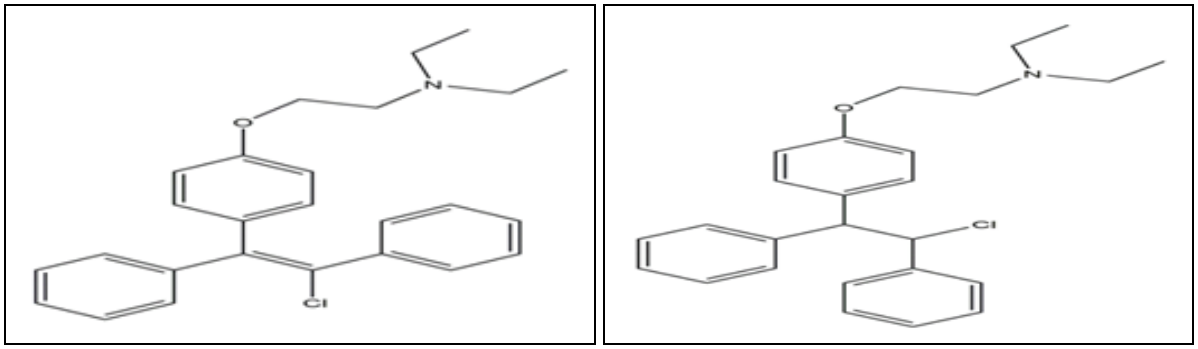
Molecular Formula: $C_{26}H_{28}CINO$, $C_6H_5O_7$

Molecular Weight: 598.1 gm

Dose: 50 mg daily for 5 consecutive days.

From the chemical point of view, clomiphene is a tri-phenylene derivative with structural similarities

to diethylboestrol. Clomiphene has two isomeric forms, cis and trans, which in the current nomenclature correspond to E-Clomiphene (enclomiphene) and Z-Clomiphene (zuclomiphene) respectively. The action of E-Clomiphene has oestrogenic, whereas Z-Clomiphene is mainly anti-oestrogenic effects. The commercial preparation is a racemic mixture that contains 60% E-Clomiphene (enclomiphene) and 40% Z-Clomiphene (zuclomiphene).



E-clomiphene enclomiphene trans clomiphene **Z- clomiphene ZOclomiphene cis clomiphene**

FIG. 1: STRUCTURE OF E-CLOMIPHENE (I) AND Z-CLOMIPHENE (II)

EXPERIMENTAL:

Materials: Clomiphene citrate standard powder was kindly supplied from Unichem Laboratories Limited, India. All chemical and reagent used were obtained from Research Laboratory, Abhilashi University and were of analytical grade.

Instrument: The instrument used for the study was a UV-Visible Spectrophotometer (Shimadzu, UV-1800, Japan) having two matched quartz cells with 1cm path length.

Preparation of Working Standard Stock Solution Preparation: 10 mg of drug was weighed accurately and 10 ml methanol was transferred into a volumetric flask and sonicated for 5-10 min. This solution was further diluted with methanol, to obtain various dilutions from 3-60 µg/ml.

Preparation of Working Standard: From above standard stock solution 5 ml was further diluted to 50 ml with Methanol followed by sonication for 5 minutes. The final strength was 100 µg/ml. The stock was used to prepare various concentration from 3-40 µg/ml by dilution with methanol.

Selection of Wavelength for Analysis of Clomiphene Citrate: Appropriate volume 1.2 ml of working stock solution of clomiphene citrate was transferred into 10 ml volumetric flask, diluted with methanol up to the mark to give a concentration 12 µg/ml. The resulting solution was scanned between 200-400 nm. Absorbance of these solutions was recorded at 294 nm & 235 nm against methanol as blank using UV-visible spectrophotometer (shown in **Fig. 2**).

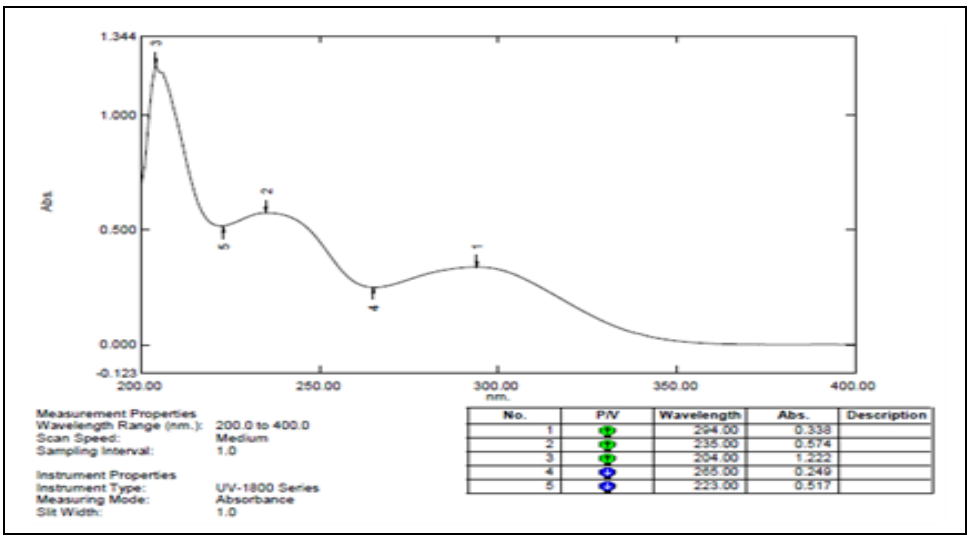


FIG. 2: ABSORPTION MAXIMA OF CLOMIPHENE CITRATE

Validation of UV Spectroscopy Method: [7-13]

Standard Calibration Curve (Linearity): A calibration curve was plotted over a concentration range of 3-40 µg/ml for clomiphene citrate at 294 nm, 3-24 µg/ml for clomiphene citrate at 235 nm. Accurately measured volume of working stock solution of clomiphene citrate at 294 nm and 235 nm were transferred to separate series of 10 ml volumetric flask and diluted up to the mark with methanol to obtain a concentration series of 3-40 µg/ml. The absorbance of all solution was taken at their respective wavelength. The calibration curve was constructed by plotting concentration against absorbance where each reading was an average of three determinations. [Shown in **Table 1, 2** and **3** and **Fig. 3a-3c, Fig. 4a-4c, Fig. 5a-5b** and **Fig. 6a-6b**].

TABLE 1: STANDARD CURVE DATA OF CLOMIPHENE CITRATE AT 294nm

Concentration µg/ml	Linearity 1 Absorbance	Linearity 2 Absorbance	Linearity 3 Absorbance
3	0.067	0.066	0.068
6	0.124	0.125	0.126
9	0.196	0.200	0.201
12	0.275	0.275	0.274
15	0.338	0.339	0.344
18	0.412	0.412	0.412
21	0.483	0.485	0.485
24	0.552	0.555	0.556
27	0.629	0.632	0.635
30	0.683	0.684	0.684
40	0.901	0.899	0.899

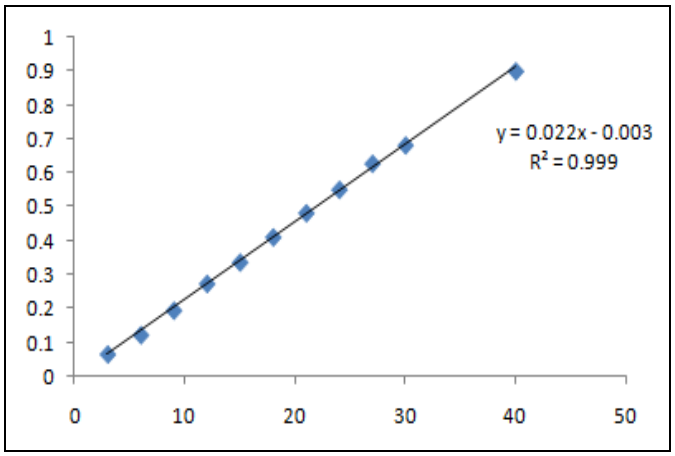


FIG. 3A: STANDARD CURVE OF CLOMIPHENE CITRATE LINEARITY 1 AT 294nm

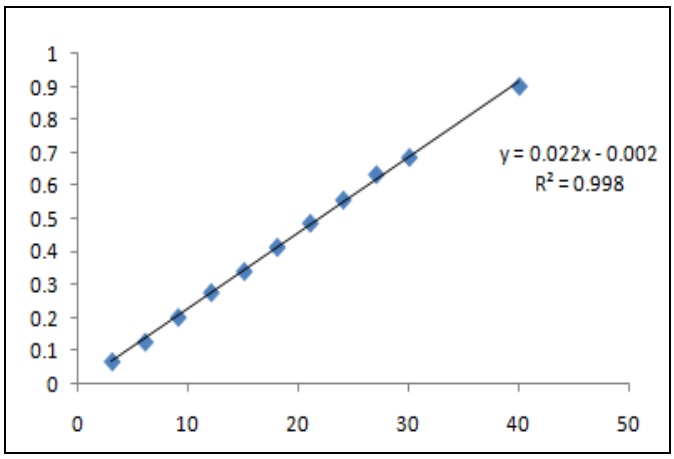


FIG. 3B: STANDARD CURVE OF CLOMIPHENE CITRATE LINEARITY 2 AT 294nm

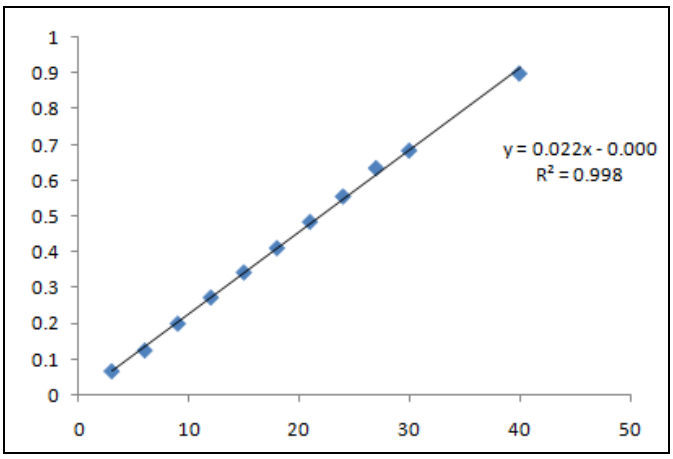


FIG. 3C: STANDARD CURVE OF CLOMIPHENE CITRATE LINEARITY 3 AT 294nm

Linearity of Clomiphene Citrate at 235nm

TABLE 2: STANDARD CURVE DATA OF CLOMIPHENE CITRATE AT 235nm

Concentration µg/ml	Linearity 1 Absorbance	Linearity 2 Absorbance	Linearity 3 Absorbance
3	0.112	0.11	0.114
6	0.208	0.209	0.211
9	0.33	0.336	0.34
12	0.468	0.467	0.467
15	0.574	0.585	0.591
18	0.703	0.704	0.705
21	0.832	0.836	0.838
24	0.946	0.952	0.956

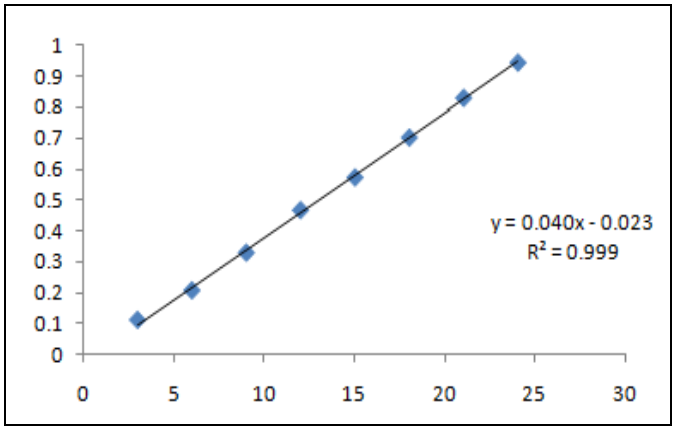


FIG. 4A: STANDARD CURVE OF CLOMIPHENE CITRATE LINEARITY 1 AT 235nm

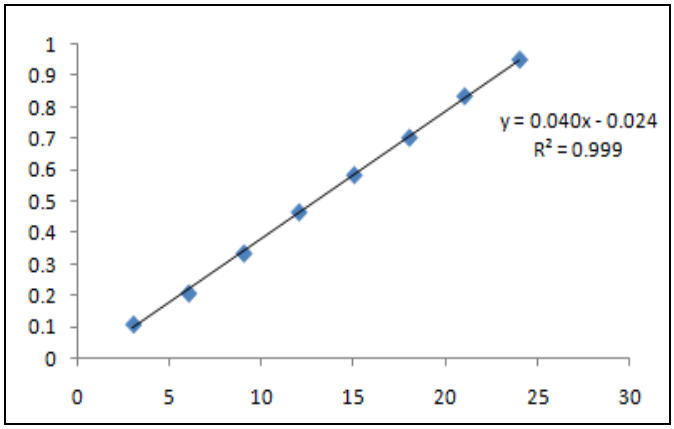


FIG. 4B: STANDARD CURVE OF CLOMIPHENE CITRATE LINEARITY 2 AT 235nm

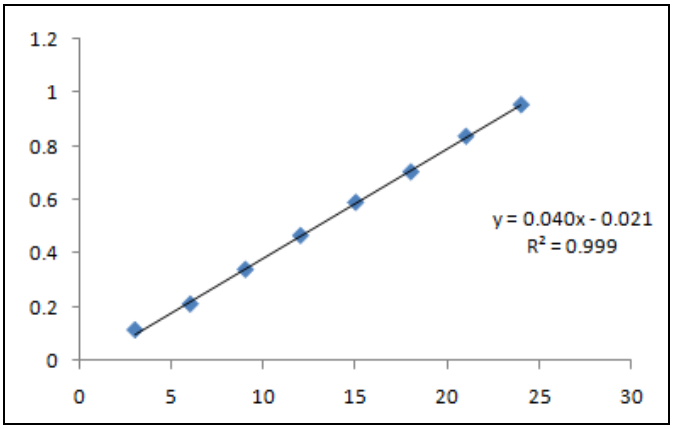


FIG. 4C: STANDARD CURVE OF CLOMIPHENE CITRATE LINEARITY 3 AT 235nm

TABLE 3: MEAN LINEARITY OF CLOMIPHENE CITRATE

S. no.	λ_{max}= 294nm		λ_{max}= 235nm	
	Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
1	3	0.067±0.001	3	0.112±0.002
2	6	0.125±0.001	6	0.209±0.002
3	9	0.199±0.003	9	0.335±0.005
4	12	0.275±0.001	12	0.467±0.001
5	15	0.340±0.003	15	0.583±0.009
6	18	0.412±0.000	18	0.704±0.001
7	21	0.484±0.001	21	0.835±0.003
8	24	0.554±0.002	24	0.951±0.005
9	27	0.632±0.003	--	--
10	30	0.684±0.001	--	--
11	40	0.900±0.001	--	--

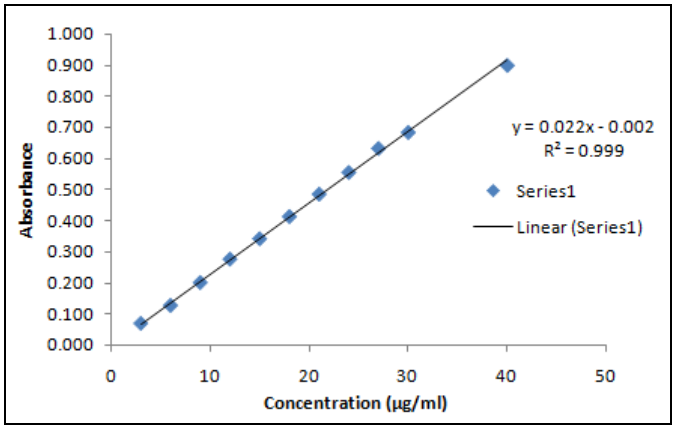


FIG. 5A: GRAPH OF STANDARD CALIBRATION CURVE OF CLOMIPHENE CITRATE IN METHANOL (λ_{max}= 294nm)

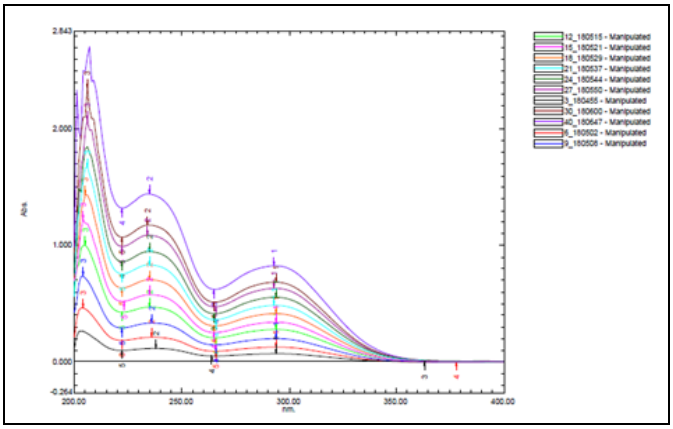


FIG. 5B: OVERLAY SPECTRUM OF CLOMIPHENE CITRATE FROM 3-40 µg/ml AT 294nm

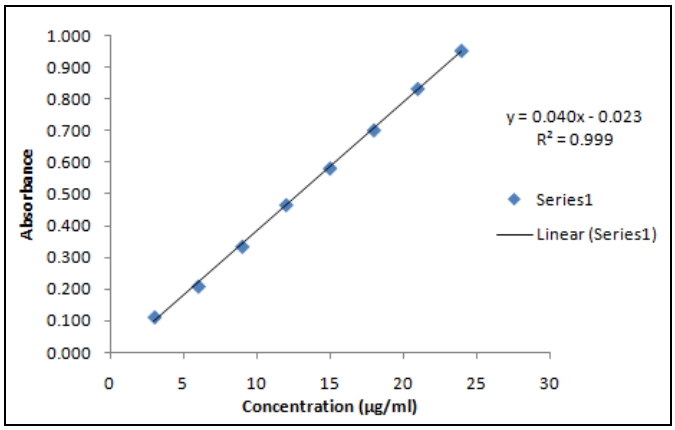


FIG. 6A: GRAPH OF STANDARD CALIBRATION CURVE OF CLOMIPHENE CITRATE IN METHANOL (λ_{max}= 235nm)

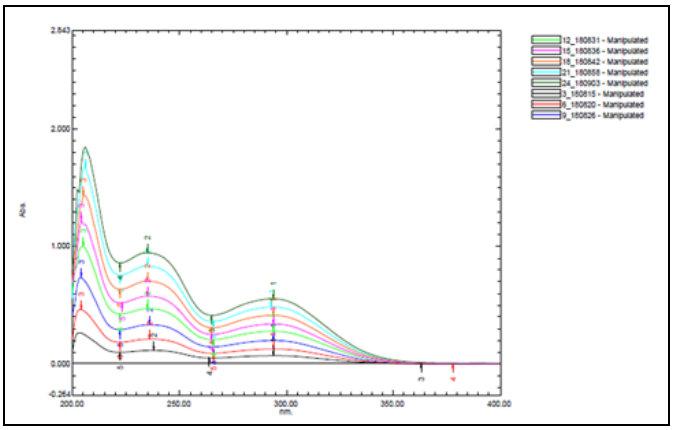


FIG. 6B: OVERLAY SPECTRUM OF CLOMIPHENE CITRATE FROM 3-24 µg/ml AT 235nm

Precision: The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation.

Repeatability: It is in the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of clomiphene citrate at 294 and 235nm (15 µg/ml) was prepared and analyzed six times as per the proposed method.

Intermediate Precision: It is the concordance of a series of measurements of the same quantity when the experiments are conducted within the same laboratory under different conditions (analyst, apparatus, instrument, and day). Standard solution of clomiphene citrate at 294 and 235nm (15 µg/ml) was prepared and analyzed as per the proposed method [shown in **Table 4**].

TABLE 4: REPEATABILITY AND INTERMEDIATE PRECISION STUDY

S. no.	Precision	Percentage recovery of Clomiphene citrate at 294nm	% RSD	Percentage recovery of Clomiphene citrate at 235nm	% RSD
1	Repeatability	103.030 ± 1.067	1.036	101.722 ± 0.981	0.965
2	Intermediate Precision (Day1-Day 3)	102.205 ± 0.921	0.901	102.065 ± 1.829	1.792

Accuracy: Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). At each level, three determinations

were performed. Percent mean recovery was calculated as shown in **Table 5** at 294 nm & 235 nm. The accepted limits of recovery are 90% - 120% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed [shown in **Table 5**].

TABLE 5: ACCURACY STUDY OF CLOMIPHENE CITRATE AT 294nm

	Clomiphene citrate at 294nm			Clomiphene citrate at 235nm		
Level	**Amount added**	**Percentage recovery**	**% RSD**	**Amount added**	**Percentage recovery**	**% RSD**
80%	12 (µg/ml)	106.439 ± 0.758	0.712	12 (µg/ml)	102.847 ± 1.867	1.816
100%	15 (µg/ml)	105.960 ± 0.763	0.720	15 (µg/ml)	101.167 ± 0.167	0.165
120%	18 (µg/ml)	99.663 ± 0.771	0.774	18 (µg/ml)	98.148 ± 1.537	1.566

Ruggedness: Ruggedness was determined by carrying out analysis by two different analysts and the respective percentage recovery was noted and the results were indicated as % RSD [shown in **Table 6**].

TABLE 6: RESULTS OF RUGGEDNESS OF CLOMIPHENE CITRATE

S. no.		**Percentage recovery of Clomiphene citrate at 294nm**	**% RSD**	**Percentage recovery of Clomiphene citrate at 235nm**	**% RSD**
1	Analyst 1	104.276 ± 1.005	0.964	102.833 ± 1.021	0.993
2	Analyst 2	106.229 ± 1.580	1.487	99.815 ± 0.933	0.934

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy (shown in **Table 7**). The LOD and LOQ of the proposed method were determined by using calibration curve:

$$LOD = 3.3\sigma / s$$

$$LOQ = 10\sigma / s$$

Where σ is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve [shown in **Table 7**].

TABLE 7: LOD AND LOQ

Drug	**LOD (µg/ml)**	**LOQ (µg/ml)**
Clomiphene citrate at 294nm	0.229	0.694
Clomiphene citrate at 235nm	0.126	0.382

Robustness: Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameter was change in wavelength. The results for robustness study in **Table 8** indicated that the small change in the conditions did not significantly affect the determination of Clomiphene citrate [shown in **Table 8**].

TABLE 8: ROBUSTNESS (CHANGE IN WAVELENGTH) FOR 15 µg/ml AT 294nm & 235nm

Conc. (µg/ml)	**Wavelength (294nm)**			**Wavelength (235nm)**		
	289nm	**294nm**	**299nm**	**230nm**	**235nm**	**240nm**
15	0.336	0.33	0.333	0.566	0.576	0.567
15	0.338	0.331	0.334	0.564	0.564	0.566
15	0.332	0.336	0.339	0.552	0.561	0.553
15	0.334	0.337	0.33	0.554	0.563	0.554
15	0.331	0.335	0.328	0.565	0.575	0.566
15	0.332	0.336	0.329	0.56	0.568	0.56
Mean	0.334	0.334	0.332	0.560	0.568	0.561
SD	0.003	0.003	0.004	0.006	0.006	0.006
%RSD	0.813	0.876	1.225	1.062	1.122	1.127

Forced Degradation Studies at 294nm & 235nm: Effect of Acid and Base Hydrolysis: Sample solution containing 1 ml aliquot of API was transferred into a 10 ml of amber volumetric flask, then mixed with 1 ml of 0.1M HCl as well as 0.1M NaOH separately and left to stand for 1 h, 2 h, 4 h at 60 °C ± 2 °C after heating on bath samples were neutralized (NaOH sample with 1 ml 0.1M HCl and HCl sample with 1 ml 0.1M NaOH) and diluted up to 10 ml with mobile phase. All three samples were injected in triplicate after the neutralizing procedure and spectra were run as described previously [shown in **Table 9** & **Fig. 7a** & **b**].

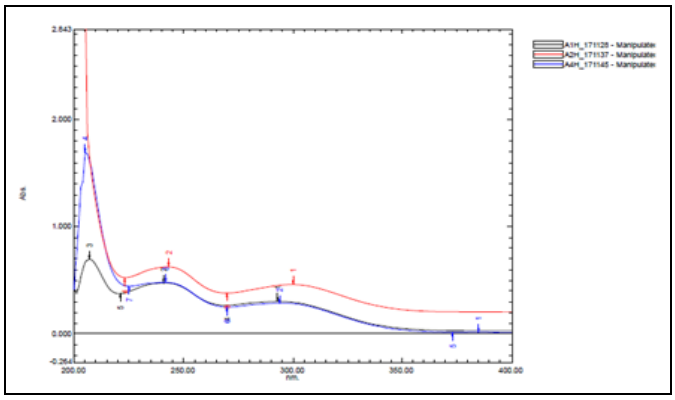


FIG. 7A: SPECTRA OF ACID DEGRADATION (0.1N HCl, HEATED FOR 1, 2 & 4 h AT 60°C)

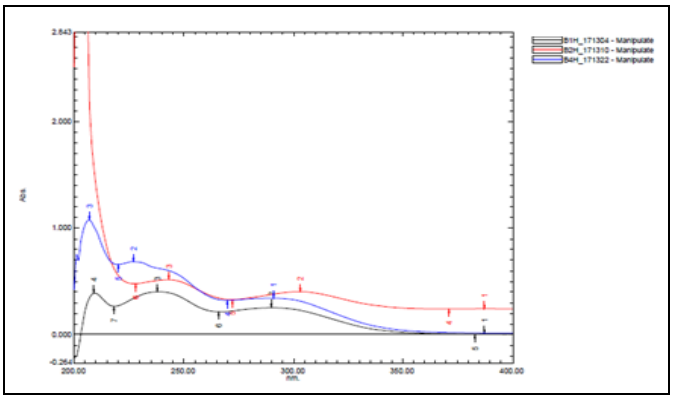


FIG. 7B: SPECTRA OF BASE DEGRADATION (0.1N NaOH, HEATED FOR 1, 2 & 4 h AT 60°C)

Effect of Oxidation: Sample solution containing 1 ml aliquot of API was transferred into a 10 ml amber volumetric flask, then mixed with 1 ml of 1% (v/v) hydrogen peroxide and left to stand for 1 hr, 2 h and 4 h at 60 °C ± 2 °C after heating on bath samples were diluted up to 10 ml with mobile phase. All three solutions were injected in triplicate and spectra were run as described previously [shown in **Table 9** & **Fig. 7c**].

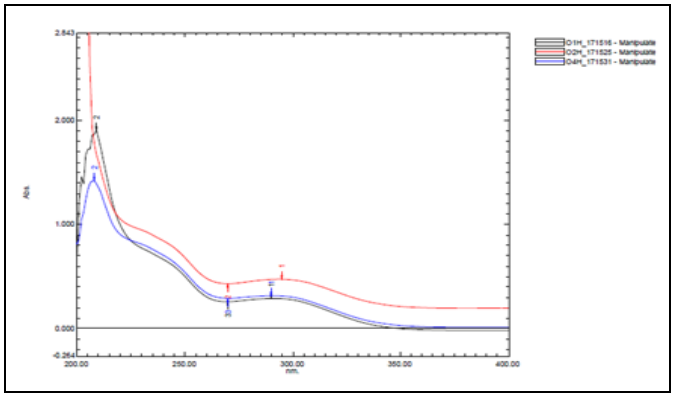


FIG. 7C: SPECTRA OF H_2O_2 (HEATED FOR 1, 2 & 4 h AT 60 °C)

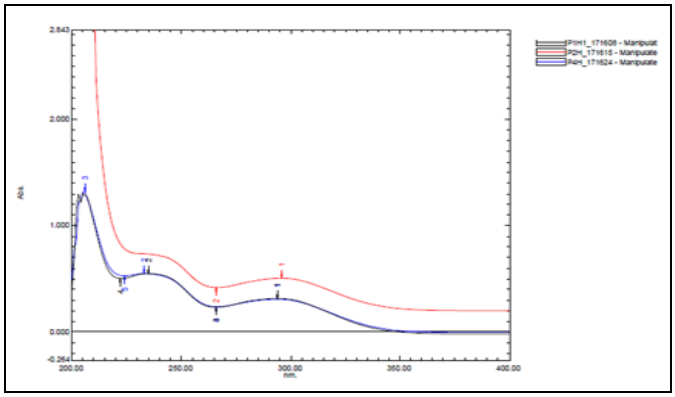


FIG. 7D: SPECTRA OF PHOTO DEGRADATION OF UV LIGHT

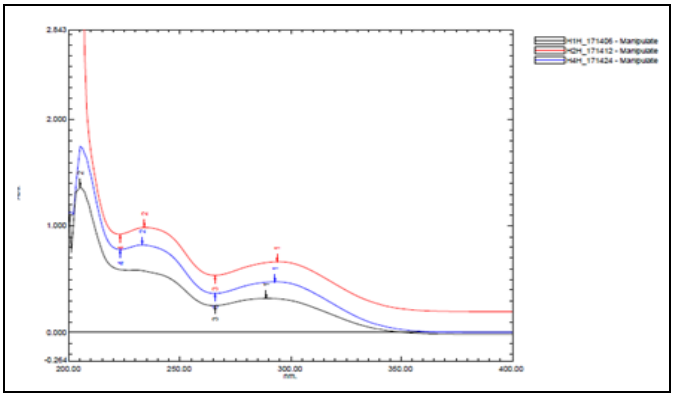


FIG. 7E: SPECTRA OF HEAT-INDUCED DEGRADATION (HEATED FOR 1, 2 &4 h AT 60°C ON WATER BATH)

Effect of Photo Degradation: Photolytic degradation was studied by placing API solution in a clear volumetric flask and exposing it to direct UV light for 1 h, 2 h and 4 h.

The resultant solution was injected in triplicate and the spectra were run as described previously [shown in **Table 9** & **Fig. 7d**].

Heat-Induced Degradation: One milliliter aliquot of a sample solution containing API was transferred to a 10 ml amber volumetric flask and then heated for 1 h, 2 h and 4 h at 60 °C ± 2 °C. The resultant each of solution were diluted in mobile phase up to 10 ml and injected in triplicate and the spectra was run as described previously [shown in **Table 9** & **Fig. 7e**].

TABLE 9: FORCE DEGRADATION STUDY AT 294 nm & 235 nm

Degradation study	Time (h)	Wavelength at 294nm		Wavelength at 235nm	
		Reported peak	Observed peak	Reported peak	Observed peak
Acid hydrolysis	1	294	293	235	241
	2	294	300	235	243
	4	294	294	235	242
Base hydrolysis	1	294	290	235	238
	2	294	303	235	243
	4	294	291	235	227
Oxidation	1	294	290	235	-
	2	294	295	235	-
	4	294	290	235	-
Photo Degradation	1	294	294	235	235
	2	294	296	235	236
	4	294	294	235	233
Heat-induced degradation	1	294	289	235	232
	2	294	294	235	236
	4	294	293	235	233

RESULTS AND DISCUSSION: The method was validated according to ICH guidelines with respect to Linearity, accuracy, precision, ruggedness, limit of detection, limit of quantitation, robustness and force degradation studies. The calibration curve for the methods were linear over concentration range 3-60 µg/ml for clomiphene citrate at 294nm, 3-24 µg/ml for clomiphene citrate at 235nm. The determination of coefficients (r^2) was 0.9991, 0.9989, 0.9988 & 0.999, 0.9994, 0.9994 respectively. The methods were found to be precision and as the % RSD value for repeatability and Intermediate day were found to be less than ±2%. The accepted limits of accuracy (recovery) were found to be 90% - 120% and all observed data are within required range which indicates good recovery value.

The result of, robustness indicted the small change in the condition did not significantly affect the determination of CC. The LOD and LOQ were found to be 0.229µg/ml, 0.694 µg/ml and 0.126 µg/ml, 0.382 µg/ml respectively**.** In forced degradation studies as acid/base hydrolysis, oxidation, photo degradation, heat-induced degradation the spectra for acid degradation of API do not showed any significant degradation or no additional peak of sample after 1 h, 2 h and 4 h at different process.

CONCLUSION: A simple, accurate, precise and cost effective UV-spectroscopic method has been developed for the estimation of clomiphene citrate. The proposed method is successfully applied for estimation of drug in any formulation. The method can be used for the routine quality control analysis of clomiphene citrate.

ACKNOWLEDGEMENT: I am thankful to my research supervisor Dr. Sachin Goyal, and research co-supervisor Dr. Amit Chaudhary. I wish to express my deep-rooted gratefulness towards, Dr. Shivali Singla for her support, edifying, enlightening discussions and timely suggestions during my research work.

It is truly said, “Teacher occupies the place of God, whose indebtedness is impossible to return”.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interests regarding the publication.

REFERENCES:

1. Kousta E, White DM and Franks S: European Society for Human Reproduction and Embryology. Human Reproduction Update 1997; 3(4): 359-65.
2. The International Pharmacopoeia, World Health Organization, Geneva, Third Edition, Vol 3, 1988: 80-82.
3. The United States Pharmacopoeia, Twenty third Edition, 1995: 400-01.
4. Martindale, The Extra Pharmacopoeia, The Pharmaceutical Press, London. 30th Edition 1993: 1179-80.
5. British Pharmacopoeia, Office of the British Pharmacopoeial Commission, London, Vol I & II. 2009: 1467-70
6. Dictionary of organic Compounds. Chapman and Hall, New York, Fifth Edition, 1982; 1: 1255.
7. International Conference on Harmonization (ICH) Guidelines Q1B, Stability Testing: Photostability Testing of New Drug Substances and Products 1996: 1-8
8. International Conference on Harmonization (ICH) Guidelines Q1A (R2), Stability Testing of New Drug Substances and Products, West ferry Circus, Canary Wharf, London 2003: 1-20
9. International Conference on Harmonization (ICH) Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 1996: 1-17
10. Iram F, Iram H, Iqbal A and Husain A: Forced Degradation Studies. J Anal Pharm Res 2016; 3(6): 00073.
11. Teunissen SF, Rosing H, Seoane MD, Brunsveld L, Schellens JHM and Schinkel AH: Investigational study of tamoxifen phase I metabolites using chromatographic and spectroscopic analytical techniques. J Pharm Biomed Anal 2011; 5(5): 518-26.
12. Vaddempudi V and Purnik SB: Development and validation of zero order spectrophotometric method for estimation of clomiphene citrate in bulk and tablet dosage form. Int Journal of Pharm Tech Res 2013; 5(1): 27-30.

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