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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CLOMIPHENE CITRATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the estimation of Clomiphene Citrate in pure and tablet forms. The method showed a linear response for concentrations in the range of 50-150 µg/mL using Acetonitrile: Phosphate Buffer solution (Dissolve 0.05 M potassium di-hydrogen orthophosphate in water.) in the ratio (60:40) as the mobile phase with detection at 245 nm and a flow rate of 1 mL/min and retention time 2.61 min. The value of correlation coefficient, slope and intercept were, 0.998, 1433 and 12098, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, oxidative, Thermal and Photolytic degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: Clomiphene Citrate, RP-HPLC, Degradation studies.

INTRODUCTION [1-10]

Stability indicating methods have become an importantaspect of any analytical method validation and a part of US FDA requirements 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 RP-HPLC. So, in the present study, a simple, precise and accurate RP-HPLC method was Developed. Clomiphene Citrate is a white or almost white powder. It is freely soluble in Acetonitrile and in Acetonitrile 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 formulations include Conductometry & UV-Visible Spectrophotometry.[4-14] The objective of the work was to develop simple, accurate, precise and economic Stability indicating RP-HPLC method development and validation for the estimation of clomiphene citrate in pharmaceutical dosage form.

MATERIALS AND METHODS

Material: 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. All reagent was obtained from Finar chem Ltd, India. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

Methods

Preparation Of Solution

- **Preparation of Mobile Phase**

⇒ Prepare 0.05M Phosphate Buffer (1.8 gm K2HPO4 in 250 ml Milli-Q-Water) then set pH 3 with OPA and Mix Acetonitrile in the ratio of 40:60 and sonicated for 20 min in ultra sonicator.

- **Preparation of Diluent**

⇒ Sonicated Acetonitrile is used as Diluent.

- **Preparation of Standard Stock Solution (1000 ppm)**

⇒ Take 100 mg of Clomiphene citrate working standard into 100 ml volumetric flask, add 25 ml of diluent, dissolve it and make up volume with diluent.

- **Standard Solution (100 ppm)**

⇒ Take 5 ml of Clomiphene citrate standard stock solution into 50 ml volumetric flask, make up volume with diluent and sonicated for 5 min in ultra-sonicator.

- **Sample Solution (100 ppm)**

⇒ Twenty tablets were accurately weighed and break it .Then transferred into 25 ml volumetric flask and 100 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. 10 ml of this solution was diluted to 100 ml with diluents. The solution was filtered through 0.45 µ Millipore PVDF filter; filtrate was collected after discarding first few ml.

Table: 1 APPARATUS AND EQUIPMENTS

Sr. No.	Instrument Name	Make and Model of Instrument
1	Electronic Analytical Balance	Shimadzu AX-200
2	HPLC	Analytical technology LTD
3	Ultra-Sonicator	PEI, Ultrasonic bath
4	Filter	0.45 µ Millipore PVDF
5	Double beam UV-Visible spectrophotometer	Shimadzu 1800
6	pH meter	Chemiline, CL-180, Labline technology PVT LTD (AL)

EXPERIMENTAL SECTION [11-14]

Calibration curves of Clomiphene citrate

The calibration curves were plotted over a concentration range of 50-150 µg/ml for Clomiphene Citrate Table No.1 and Figure No.2. Accurately measured standard solutions of Clomiphene Citrate (5, 7.5, 10, 12.5, 15 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with Acetonitrile. The calibration curves were constructed by plotting area versus concentrations and the regression equations were calculated.

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 Acetonitrile, mechanically shaken for 10 min, ultrasonicated for 5 min, and then diluted to volume with Acetonitrile.

RESULTS AND DISCUSSION

Forced degradation study

Acid Degradation: Take 10 ml of Clomiphene Citrate from Standard stock solution into 100 ml of volumetric flask; add 1.0 ml of 1N HCl, kept at room temp. for 24 hr., after that neutralized with 1 ml of 1N NaOH solution, shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

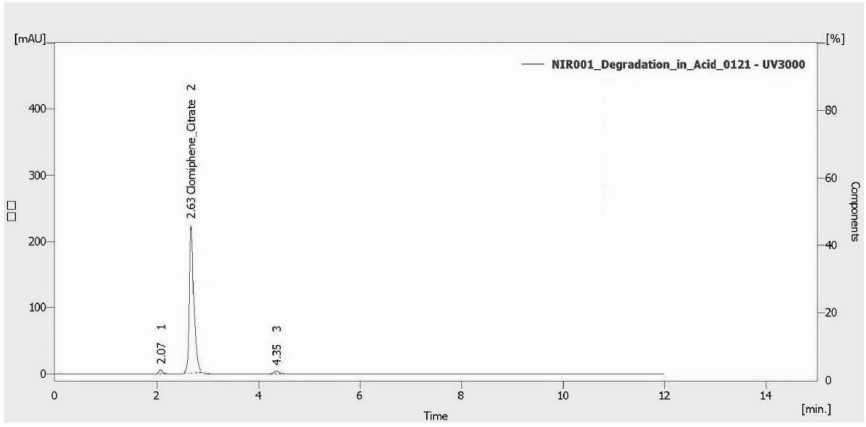

Fig 1: Chromatogram of Acid degradation

Alkali Degradation: Take 10 ml of Clomiphene Citrate from Standard stock solution into 100 ml of volumetric flask; add 1.0 ml of 1N NaOH, kept at room temp. for 24 hr., after that neutralized with 1 ml of 1N HCl solution, shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

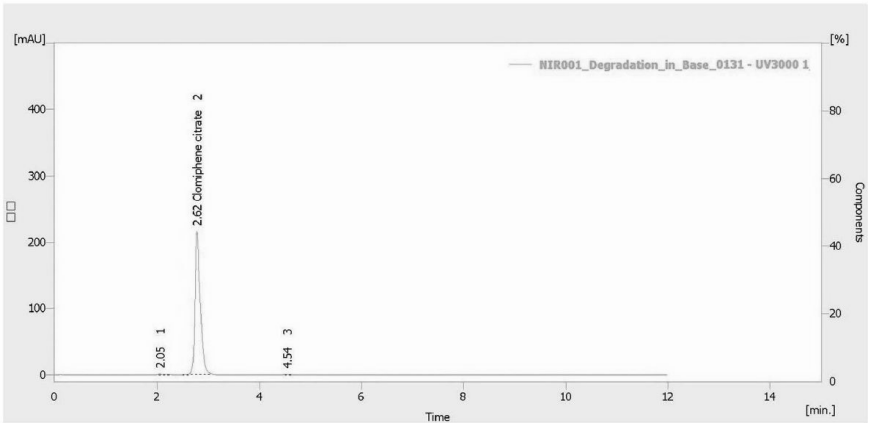

Fig 2: Chromatogeam of Base Degradation

Oxidative Degradation: Take 10 ml of Clomiphene Citrate from Standard stock solution into 100 ml of volumetric flask; add 1.0 ml of 30% H_2O_2 kept at room temp. for 24 hr, shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

- **Photolytic Degradation:** Take 10 ml of Clomiphene Citrate from Standard stock solution into 100 ml of volumetric flask and kept in U.V chamber 24 hr., after that shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.
- **Thermal Degradation:** Take 10 ml of Clomiphene Citrate from Standard stock solution into 100 ml of volumetric flask and kept in 70ºC for 24 hr., after that shake solution for 5 min., make up volume up to the mark with diluent, and then go for HPLC analysis.

◆ **Chromatographic conditions**

⇒ **Selection of Stationary Phase:**On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C18 bonded phase i.e. Thermo cyano C18 (25 X 4.6 mm) 5µ was selected.

o **Selection of Mobile Phase**

⇒ After assessing the solubility of drug in different solvent as well on the basis of literature survey; the mobile phase was selected is the mixture of Acetonitrile: 0.05M Phosphate buffer (60:40) & P^H was set to 3 by Ortho phosphoric Acid.

o **Selection of Detector and Detection Wavelength**

⇒ UV detector was selected, as it is reliable and wavelength was set to 245 nm.

Table 2: Result of System Suitability for Assay Method

Theoretical plate	2994
Tailing Factor	1.3594
Retention time	2.63 (Min.)

Table 3: Summary of Force degradation study of Clomiphene Citrate

Sr No.	Condition	% Degradation	Remark
1	Acid Degradation (1N HCl)	5.92	Degradation observed
2	Alkali Degradation (1N NaOH)	8.75	Degradation observed
3	Peroxide Degradation (30% H2O2) v/v	1.63	Less Degradation observed
4	Thermal Degradation (70^oC)	2.13	Less Degradation observed
5	Photolytic Degradation	1.31	Less Degradation observed

Stability indicating assay: Degradation was observed for Clomiphene citrate sample during stress conditions like acid, alkaline, Oxidative, Photolytic, and in thermal condition. Graph

shows test results confirmed Clomiphene citrate peak is homogeneous in all the stress conditions tested.

Sample Chromatogram

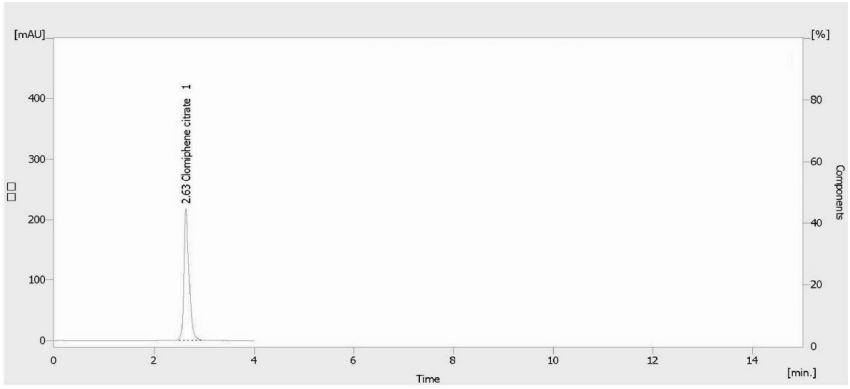

Fig 3: Chromatogram of Standard Clomiphene Citrate

❖ **VALIDATION OF METHOD**

- **Linearity, LOD & LOQ**

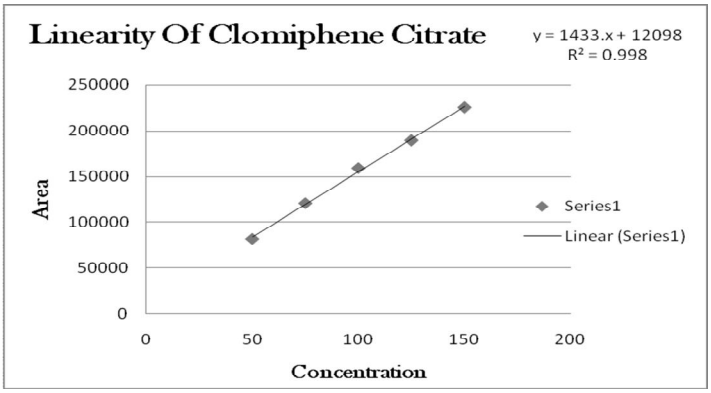

Fig 4: Calibration Curve of Clomiphene citrate (50 – 150 ppm) for Assay Method

Table 4: Result of Linearity, LOD and LOQ for Assay Method

Sr No.	**Conc. (ppm)**	**Mean Area (mAU*S)**
1	50	81490.558
2	75	120628.415
3	100	159227.421
4	125	189730.716
5	150	226115.044
Mean Intercept	12098	
Mean Slop	1433	
r^2	0.998	
LOD	0.013 mcg/ml	**LOQ** 0.038 mcg/ml

* Avg. of 3 determination

❖ **ACCURACY**

Table 5: Result of Accuracy for Assay Method

Spiked level (%)	Conc. in sample (µg/ml)	Conc. added in (µg/ml)	Total conc. (µg/ml)	Conc. Recovered (µg/ml)	% Recovery	SD	% RSD
80	65	52	117	116.00	99.14	0..0269	0.0271
80	65			115.49	98.70		
80	65			116.06	99.19		
100	65	65	130	129.70	99.76	0.496	0.494
100	65			130.97	100.74		
100	65			130.51	100.39		
120	65	78	143	143.79	100.56	0.665	0.666
120	65			141.99	99.29		
120	65			142.4	99.58		

❖ **PRECISION**

➢ **Method Precision (Repeatability)**

Table 6:- Result of Method Precision (Repeatability) for Assay Method

Sr. No.	Peak Area (mAU*S)	Mean Area (mAU*S)	SD	% RSD
1	158021.995	**158276.6477**	**236.712**	**0.149**
2	158224.892			
3	158101.609			
4	158635.353			
5	158489.341			
6	158186.696			

❖ **Intermediate Precision**

❖

Table 7: Result of Intermediate Precision for Assay Method

Day	Conc. (ppm)	Area (mAU*S)	Mean	SD	% RSD
1	100	155137.362	**155215**	**94.538**	**0.060**
2	100	155254.373			
3	100	155390.111			
4	100	155158.197			
5	100	155173.043			
6	100	155176.810			

ROBUSTNESS

Change in Flow rate (± 0.2 ml/min)

Table 8: Result of Change in Flow Rate for Assay Method

Sr. No.	Flow rate 1.2 ml/min (+0.2ml/min) Area (mAU*S)	Flow rate 0.8 ml/min , (- 0.2 ml/min) Area (mAU*S)
1	144952.145	161976.139
2	145341.127	162471.257
3	145054.179	163621.169
4	145423.256	161136.173
5	145945.324	161931.151
6	146342.237	162973.273
Mean	145509.7	162351.5
% RSD	0.004	0.51

ASSAY OF CLOMIPHENE CITRATE TABLET

Table 9:- Result of Assay of Clomiphene Citrate Tablet by Assay Method

Sr No.	Area (mAU*S)	% Assay (n = 3)
1	155155.098	97.35 %
2	155761.084	97.73 %
3	156379.078	2%

SUMMARY & CONCLUSION

Table 10: Summary of Validation of Assay Method

Sr. No.	Test	Limit	Results
1	Specificity	A) Placebo Interference	No Interference
2	System suitability	% RSD NMT 2.0	% RSD =0.0659
3	Linearity	r^2 NLT 0.999	r^2 0.9989
4	Accuracy	% RSD NMT 2.0	0.396
5	Method Precision (Repeatability)	% RSD NMT 2.0	0.149
6	Intermediate Precision	% RSD NMT 2.0	0.060
7	Robustness	% RSD NMT 2.0	0.351
8	LOD	-	0.013 mcg/ml
9	LOQ	-	0.038 mcg/ml

CONCLUSION

- A simple, precise and accurate analytical simple and stability indicating RP-HPLC methods has been developed for estimation of Clomiphene citrate drug in Tablet formulation.
- The developed methods have been validated as per ICH guidelines, and it meets all the acceptance criteria given in ICH guidelines.
- The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time. The results of forced degradation within the acceptance criteria. So that simple assay method could be used for the routine analysis of Clomiphene citrate.

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