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Simultaneous equation method for the estimation of Clomiphene citrate and Acetylcysteine by UV-Visible Spectrophotometry.

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ABSTRACT:
The current study describes a simple, accurate, and exact UV spectrophotometric method for estimating Clomiphene citrate and Acetylcysteine in tablet dosage form simultaneously. Clomiphene citrate and Acetylcysteine absorbance was measured at two wavelengths, 232 nm and 219 nm, respectively. Isobestic point was found to be 225nm. Clomiphene showed linearity in the range of 2µg/ml to 10µg/ml (r^2=0.998) and Acetylcysteine in the range of 24µg/ml to120µg/ml (r^2=0.996). Clomiphene citrate had a percentange mean recovery of 100.51 %. While Acetyl cysteine had a percentange mean recovery of 103.23 %. . The recovery study's percentage RSD was less than 2. The methodologies were validated in accordance with the ICH recommendations.

Keywords: Clomiphenecitrate ; Acetylcysteine ; Simultaneous equation; Validation; UV spectrophotometer.

I. INTRODUCTION:

Acetylcysteine, also known as N-acetylcysteine, N-acetyl-L-cysteine, or NAC, is made from cysteine by adding an acetyl group to the amino group. N-Acetyl cysteine is an active pharmaceutical ingredient and dietary supplement that is primarily utilized as a mucolytic and in the treatment of paracetamol overdose. Acetylcysteine is an antioxidant in and of itself, but it is also deacetylated to cysteine, a component of the antioxidant glutathione production. If you're allergic to Acetylcysteine or any other component of the Acetylcysteine solution, don't take it[1,3.]

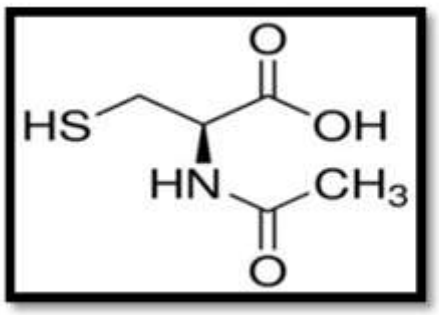

Figure1: Structure of Acetylcysteine

Ethanamine, 2-[4- (2- chloro-1, 2-diphenyl-ethenyl)-phenoxy l-N, N-diethyl-, 2-hydroxy-1,2,3 propane tricarboxylate] is another name for clomiphene citrate. This is an on-steroidal compound. Clomiphene citrate has be used to stimulat eovulation since 1962 . That is the situation. Women with polycystic ovaries are disproportionately affected by this first-line medication for ovulatory infertility in women with illnesses that are oestrogenized naturally (PCO). Both oestrogenic and anti-estrogenic characteristics are found in Clomiphene citrate. Characteristics that are oestrogenic and anti-estrogenic having a deterring effect. The oestrogen hypothalamus and endogenousoe strogen sites of pituitary oestrogen receptors are thought to be displaced by Clomiphene citrate. Insulin-sensitizing drugs have been investigated for treating the underlying cause of illnesses linked with insulin resistance, and the discrepancy may continue to some extent with gonadotropin treatment[4.]

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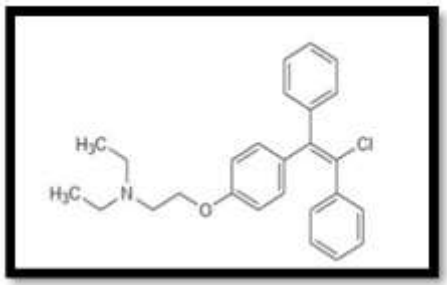


Figure2: Structure of Clomiphene citrate

From literature survey it was found that no any UV method has been reported on this combination respectively. In this present research work, it was proposed to developed and validate a new, simple, and accurate UV method for simultaneous estimation of Clomiphene citrate and Acetylcysteine in marketed dosage formulations[5].

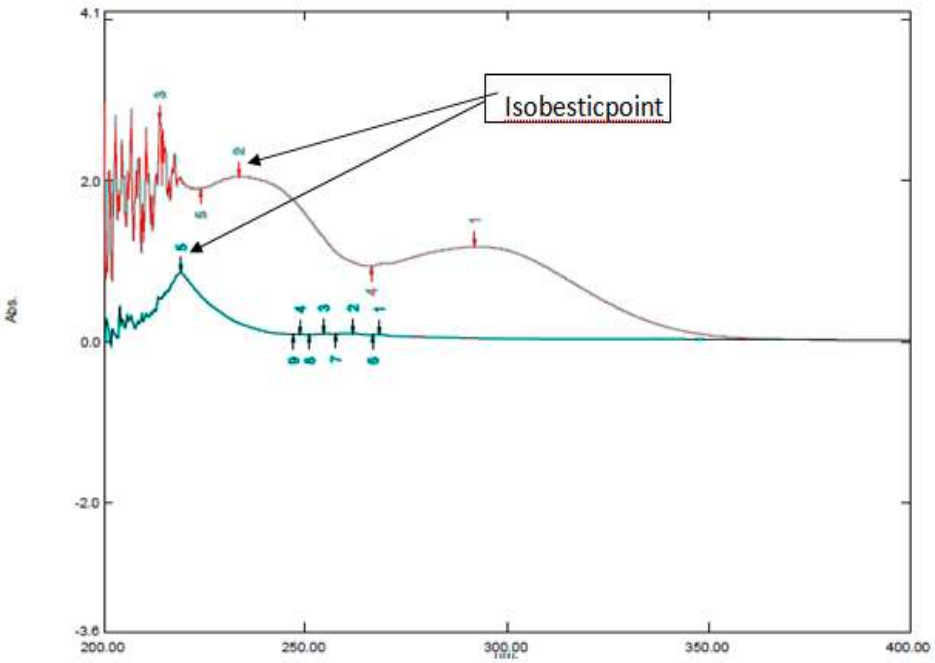
II. MATERIAL AND METHOD:

2.1 Chemicals and Reagents :

Analytical pure sample of Clomiphene citrate and Acetylcysteine were received as a gift sample from Lupin Limited M. I. D. C, Tarapur via Boisar were used in the study.The pharmaceutical dosage form used in this study was "U MOM" labeled to contain Acetylcysteine and Clomiphene citrate 600/50 mg per tablet. The solvent used were of Methanol and Distilled water used in preparation of mobile phase.

2.2 Selection of wavelength :

UV-spectrum of Clomiphene citrate and Acetylcysteine at 232nm and 219nm respectively. Mobile phase Methanol : Water (80:20%v/v) is used for this good peaks, good absorbance and better sensitivity. Both drugs absorbed at same point shown in figure 3.



Isobestic point

Figure 3: Isobestic point of Clomiphene citrate & Acetylcysteine

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2.3 Instrument used :

A Shimadzu 1800 UV/VIS double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements[6.]

2.4 Preparation of Mobile phase :

1000ml mobile phase was prepared by mixing 800ml methanol and 200ml distilled water (80:20%v/v).

2.5 Preparation of stock solution of Clomiphene citrate :

Prepare a standard stock solution of Clomiphene citrate by adding 50 mg in 50 ml volumetric flask & make the volume to 50 ml with diluent. Then pipette out 0.1ml and add 10 ml volumetric flask and make the volume again 10ml with diluent. (Concentration of Clomiphene citrate = 10µg/ml).

2.6 Preparation of stock solution of Acetylcysteine :

Prepare a standard stock solution of Acetyl cysteine by adding 50mg in 50ml volumetric flask & make the volume to 50 ml with diluent. Then pipette out 1.2 ml and add 10 ml volumetric flask and make the volume again10ml with diluent. (Concentration of Acetylcysteine = 120µg/ml).

2.7 Simultaneous estimation of Clomiphene citrate and Acetylcysteine :

In simultaneous method, we used absorbance at two selected wavelengths. To determine the λmax of both the drugs we scan in the range of 200-400nm . Standard solutions of different concentrations of both drugs were prepared in mobile phase. Absorbance of Clomiphene (10µg/ml) and Acetylcysteine (120µg/ml) were recorded at two wavelengths 232nm and 219 nm by using simultaneous equation method[7,9.]

$$Cx=A2ay1-A1ay2/ax2ay1-ax1ay2$$

$$Cy=A1ax2-A2ax1/ax2ay1-ax1ay2$$

Cx=concentration of Clomiphene citrate
Cy=concentration of Acetylcysteine
ax1and ax2= absorptivity value of Clomiphene citrate
at 232nm and 219 nm
ay1anday2= absorptivity value of Acetylcysteine at 232 nm and 219 nm
A1=absorbance of standard mixture at 232nm
A2=absorbance of standard mixture at 219nm

2.8 Analysis of marketed formulation:

Five tablets of brand name "U MOM" were used. From the five tablets accurately weighed the powder equivalent to single tablet (Clomiphene citrate 50mg and Acetyl cysteine 600mg) 50 mg Clomiphene citrate and 50 mg of Acetyl cysteine were transferred into a 50 ml volumetric flask and 50 ml solvenet was added and sonicator approximately for 10 min. then passed it through the Whatman filter paper and make up volume up to 50 ml from solvent take 0.1 ml of above filtrate was transferred into 10 ml volumetric flask and the final volume was adjusted upto the mark with same solvent to get the sample solution with the concentration of 10µg/ml Clomiphene citrate and also take 1.2ml above filtrate was transferred into10ml volumetric flask and make the volume Again 10ml with solvent to get the solution with the conc. Of Acetylcysteine 120µg/ml respectively[10,15.]

Table1:Analysis of marketed formulation

Sr.No	Clomiphene citrate		% Recovery	Acetylcysteine		% Recovery
	Absorbance	Amount recoveredµg/ml		Absorbance	Amount recovered µg/ml	
1	0.290	9.76	97.6	0.333	61.09	101.8
2	0.293	9.86	98.6	0.336	61.42	102.3
3	0.295	9.93	99.3	0.332	60.77	101.2

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4	0.297	10	100	0.339	61.26	102.1
5	0.294	9.9	99	0.341	60.93	101.5
Mean	**0.293**	**9.89**	**98.9**	**0.336**	**61.09**	**101.8**
%RSD	**0.8810**	**0.8987**	**0.8987**	**1.1404**	**0.4218**	**0.4218**

III. METHOD VALIDATION :

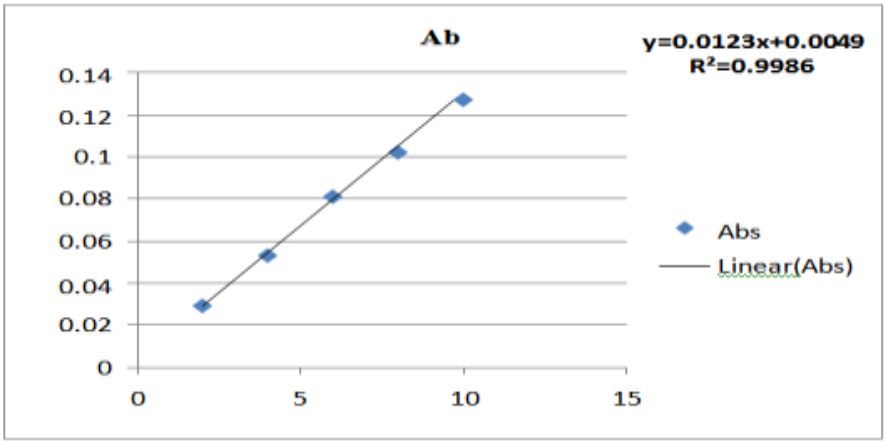
Validation of an analytical method is the process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application. The UV method was validated in terms of linearity, accuracy, precision, LOD and LOQ[17,18.]

3.1 Linearity:

Linearity was studied by plotting a graph of absorbance is directly proportional to the concentration. A series of standard solution of Clomiphene citrate were prepared in the concentration range of about 2 µg/ml to 10 µg/ml and Acetyl cysteine concentration range is 24µg/ml to 120 µg/ml is shown in below table (2).The linearity graph of Clomiphene citrate and Acetylcysteine shown in fig.no.4&5.

Table 2:Linearity study of Clomiphene citrate and Acetylcysteine

Srno.	Concentratin (µg /ml) of Clomiphene citrate	Concentratin (µg /ml) of Acetylcysteine	Absorbance of Clomiphene citrate at 232 nm	Absorbance of Acetylcysteine at 219nm
1	2	24	0.029	0.322
2	4	48	0.053	0.421
3	6	72	0.081	0.523
4	8	96	0.102	0.594
5	10	120	0.127	0.711



Concentration (µg/ml)

Fig4: Linearity graph of Clomiphene citrate

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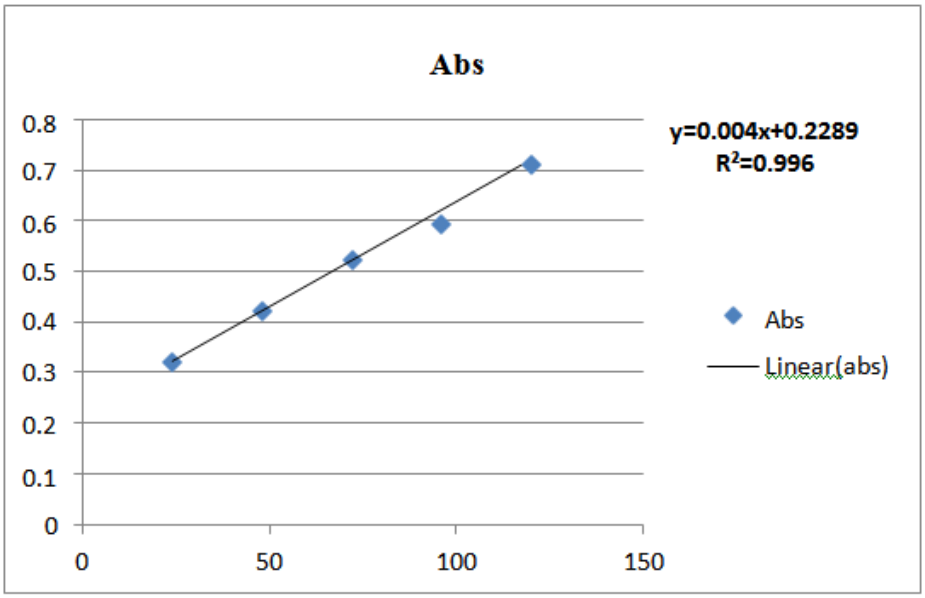

Concentration(µg/ml)
Fig5:Linearity graph of Acetylcysteine

3.2 Precision:

Six separate solutions comprising concentrations of 2,4, and 6 µg/ml of Clomiphene citrate and 24, 48, and 72 µg/ml of Acetylcysteine were analysed for repeatability. The absorbance was measured three times in each day to determine intra-day and inter-day variation. The %RSD was determined to be less than 2in the tables below 3, 4, 5, 6)

Table3: Intra-day precision of Clomiphene citrate

Conc.µg/ml	Absorbance			Mean	SD	%RSD
	Trial1	Trial2	Trial3			
2	0.182	0.184	0.183	0.183	0.001	0.0546
4	0.362	0.368	0.371	0.367	0.00458	1.2487
6	0.413	0.412	0.413	0.412	0.00058	0.1401

Table4: Intra-day precision of Acetylcysteine

Conc.µg/ml	Absorbance			Mean	SD	%RSD
	Trial 1	Trial 2	Trial 3			
24	0.321	0.329	0.322	0.324	0.00435	1.3453
48	0.413	0.418	0.418	0.415	0.00251	0.6065
72	0.529	0.531	0.532	0.530	0.00142	0.2883

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Table 5:Inter-day precision of Clomiphene citrate

Conc.µg/ml	Absorbance			Mean	SD	%RSD
	Trial 1	Trial 2	Trial 3			
2	0.181	0.183	0.183	0.182	0.00115	0.6346
4	0.361	0.368	0.369	0.366	0.00436	1.1909
6	0.412	0.409	0.411	0.410	0.00153	0.3726

Table 6: Inter-day precision of Acetylcysteine

Conc.µg/ml	Absorbance			Mean	SD	%RSD
	Tria 1	Trial 2	Trial 3			
24	0.321	0.323	0.321	0.321	0.00115	0.3598
48	0.411	0.412	0.421	0.414	0.00551	1.3304
72	0.522	0.531	0.530	0.527	0.00493	0.9361

3.3 Accuracy:

This parameter is performed to determine the closeness of the test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (50%,100%,and150%) and the % recovery of Clomiphene citrate and Acetyl cysteine was calculated below table no. (7 &8)

Table 7: Recovery study of Clomiphene citrate

Level	Conc.(µg/ml)		Absorbance	%Recovery
	Sample	Standard		
50%	4	2	0.476	101.51
100%	4	4	0.621	99.67
150%	4	6	0.786	101.16
Mean 100.78				

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Table7: Recovery study of Acetylcysteine

Level	Conc.(µg/ml)		Absorbance	%Recovery
	Sample	Standard		
50%	48	24	0.997	103.78
100%	48	48	1.256	102.36
150%	48	72	1.542	103.56
Mean 103.2				

3.4. Robustness:

The analytical technique's robustness is a measure of it's ability to remain unaffected by tiny but deliberate modifications in the method parameters, and it gives an indicator of its depend ability in routine use. For Clomiphene citrate and Acetylcysteine, the method's robustness was investigated.

3.5. Sensitivity:

The limit of detection [LOD] and limit of Quantitation [LOQ] parameters were calculated using following equations;

$$LOD=3.3\sigma/S$$

And

$$LOQ=10\sigma/S$$

Where,

σ=Standard deviation of y-Intercept of regression line.

S=Slope of the calibrationcurve.

3.6. Limit of Detection (LOD) and Limit of Quantitation (LOQ) Determination:

Limit of Quantitation is 3 times more than the limit of detection resp. The LOD value of Clomiphene citrate and Acetyl cysteine is 10.6 µg/ml and 124.2 µg/ml respectively and the LOQ value were found to be 32.3µg/ml and 376.6µg/ml Clomiphene citrate and Acetylcysteine.

Table 9: Result of LOD AND LOQ

Sr no.	Name of drugs	LOD(microgram/ml)	LOQ (microgram/ml)
1	Clomiphene citrate	10.6	124.2
2	Acetylcysteine.	32.3	376.6

IV. RESULT AND DISCUSSION:

The proposed method is based on spectrophotometric simultaneous estimation of Clomiphene citrate and Acetylcysteine in this method methanol and distilled water is used as solvent. The calibration plot for the method was linearity range concentration of 24 to 120µg/ml for Acetyl cysteine and 2 to 10 µg/ml for Clomiphene citrate respectively. The determination of coefficients (r^2) was 0.996and0.998 for Acetylcysteine and Clomiphene citrate respectively.

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Isobestic point was found to be 225 nm .The method was found to be precise and as the % RSD values for intra-day and inter- day were found to be less than 2% for Acetylcysteine and Clomiphene citrate respectively. The LOD and LOQ were found to be 124.2 µg/ml and 376.6µg/ml for Acetylcysteine and 10.6µg/ml and 32.3µg/ml for Clomiphene citrate respectively.The percentange mean recovery was found to be 100.3 % for Clomiphene citrate and 103.2 % for Acetylcysteine. There sults of assay showed that the amount of drug as indicated by % assayfor104.7 % Acetyl cysteine and 103.3% for Clomiphene citrate. The proposed method was also successfully applied to a pharmaceutical formulation[18,19.]

V. CONCLUSION:

Our findings show that the proposed UV spectroscopic approach is straight forward, quick, precise and accurate. Without interference from excipients, the established UV spectroscopic methods were proven to be acceptable for determining Clomiphene citrate and Acetylcysteine as bulk drug and in marketed solid dosage formulations. These procedures are reproducible and selective for the measurement of Clomiphene citrate and Acetyl cysteine, according to statistical analysis.

VI. ACKNOWLEDGEMENT:

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Conflict of interest:

The authors declare that the reisno conflict of interest.

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