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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF UBIDECARENONE (COENZYME Q-10) AND CLOMIFENE CITRATE IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

In the present work two new, simple, accurate and precise UV Spectrophotometric methods have been developed and validated for the simultaneous estimation of Ubidecarenone and Clomifene Citrate in combined tablet dosage forms. Method –I involves formation and solving the simultaneous equation by using two selected wavelengths 275.0 nm and 296.0 nm, the λ_{\max} of Ubidecarenone and Clomifene Citrate respectively. Method- II is Absorbance ratio/ Q- Analysis method based on the measurement of absorptivity value of two drugs at 282.3 nm (Isoabsorptive point) and 296.0 nm (λ_{\max} of Clomifene Citrate). The Linearity was observed in the range of 12-36 $\mu\text{g/ml}$ and 10-30 $\mu\text{g/ml}$ for Ubidecarenone and Clomifene Citrate respectively. The accuracy and precision of the methods were determined and validated. The percentage recovery of the drugs by the developed method was found to be in the ranges of from 98.0% - 102.8%, that indicates the good accuracy of the method. The developed methods can be used for the routine analysis for the estimation of Ubidecarenone and Clomifene Citrate in pharmaceutical formulations.

KEY WORDS

Ubidecarenone, Clomifene Citrate, Simultaneous equation method and Q-Absorbance ratio method.

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INTRODUCTION

Ubidecarenone is chemically 2-[(all-E)-3,7,11,15, 19,23,27,31,35,39- Decamethyltetraconta 2,6,10,14, 18,22,26,30,34,38 –decaenyl]-5, 6 dimethoxy, 3-methyl benzene-1,4-dione, which is also called as coenzyme Q10. It is used as a dietary supplement and categorized as a cardio vascular agent used in the treatment of congestive heart failure and angina pectoris. It is official in BP, USP, and JP. Ubidecarenone alone (or) in combined formulation

with other drugs is reported to be estimated by HPLC and UV/VIS Spectrophotometric methods¹. Clomifene Citrate is chemically known as Ethanamine, 2-[4-(2-chloro-1, 2-diphenylethenyl) phenoxy]-N, N-diethyl-, 2-hydroxy-1, 2, 3-propanetricarboxylate (1:1).2- [p-(2- Chloro-, 2 diphenylvinyl) phenoxy] triethylamine Citrate and it is used in the management of infertility in normally oestrogenized, anovulatory women. Clomifene Citrate is estimated in pure sample by using non aqueous titration method and UV/VIS Spectrophotometric method is used to estimate in the formulated product². These two methods are mentioned in Japanese pharmacopoeia edition 2009³. Literature review revealed that several methods have been reported for the quantification of Ubidecarenone and Clomifene Citrate individually. However there is no UV/VIS Spectrophotometric methods have been reported for the simultaneous estimation of Ubidecarenone and Clomifene Citrate in Pharmaceutical formulations. The present paper describes a simple, rapid, economical and accurate method developed and validated for the estimation of Ubidecarenone and Clomifene Citrate simultaneously⁴.

MATERIALS AND METHOD

Shimadzu double beam UV/Visible spectrophotometer (Model- UV 1800) with spectral band width of 2 nm and a pair of 10 mm matched quartz cuvettes were used for all absorbance measurements. Pure drug sample of Ubidecarenone, Clomifene Citrate and commercial formulation of Ubidecarenone and Clomifene Citrate (Ubiphene) were obtained as a gift sample from M/s. Fourrts (India) Laboratories, Chennai. All chemicals and reagents used were of analytical grade.

Preparation of Standard Stock Solution

Standard stock solution of Ubidecarenone and Clomifene Citrate were prepared separately by dissolving 24 mg of Ubidecarenone and 20mg of Clomifene Citrate in 1ml of Hexane. Further dilution is made with 25%v/v Ethanol in Methanol to get the concentration of 24 µg/ml of Ubidecarenone and 20 µg/ml of Clomifene Citrate respectively⁵. The

resulting solution was then scanned in the UV range of 200 nm-400 nm in 1cm quartz cell against the solvent blank and the overlain spectrum of Ubidecarenone and Clomifene Citrate was recorded.

Method-I Simultaneous equation method

Standard stock solution of Ubidecarenone and Clomifene Citrate were diluted separately to get the series of concentration of 12-36 µg/ml of Ubidecarenone and 10-30 µg/ml of Clomifene Citrate respectively and scanned in the UV range of 200 nm-400 nm. Ubidecarenone shows the maximum absorbance (λ_{max}) at 275.0 nm and Clomifene Citrate shows the maximum absorbance at 296.0 nm. These two wavelengths were selected to framing the simultaneous equation to find out the concentration of the drugs individually in the mixed wavelengths and the absorptivity values of Ubidecarenone and Clomifene Citrate were determined. The concentration of each drug was estimated by substituting the absorbance and absorptivity value in the following equations⁷.

$$A_1 = 0.01570 C_1 + 0.01625 C_2 \text{ ----- [1]}$$

$$A_2 = 0.00237 C_1 + 0.01900 C_2 \text{ ----- [2]}$$

Where, A_1 and A_2 are the absorbance of sample at 275.0.0 nm and 296.0 nm respectively

C_1 and C_2 are the concentration of UBI and CLO in µg/ml respectively.

By rearranging the equation 1 and 2 as per the Cramer's rule, the concentration of Ubidecarenone and Clomifene Citrate can be calculated as follow as,

$$C_{UBI} = \frac{A_2(0.01693) - A_1(0.01900)}{-0.000270} \text{ - [3]}$$

$$C_{CLO} = \frac{A_1(0.00237) - A_2(0.01541)}{-0.000270} \text{ - [4]}$$

Method- II Absorbance Ratio / Q- Analysis Method

In this method, two wavelengths were selected, one at 282.3 nm (Isoabsorptive point) and another one at 296.0 nm (λ_{max} of Clomifene Citrate).The working standard solution of Ubidecarenone (24 µg/ml) and Clomifene Citrate (20 µg/ml) were prepared

separately as per the procedure mentioned above and the absorbance were measured in UV/Vis spectroscopy against the solvent blank⁸. Both drugs were found to have the same absorbance at the wavelength 282.3 nm (Isoabsorptive point). The absorptivity value of both drugs was calculated from the measured absorbance. The concentration of Ubidecarenone and Clomifene Citrate was calculated by the following equations^{9,10}.

$$C_{\text{UBI}} = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A_1}{aX_1} \quad \text{--- [5]}$$

$$C_{\text{UBI}} = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{A_2}{aY_1} \quad \text{--- [6]}$$

Where, A1 and A2 are the absorbance of sample at 282.3 nm and 296.0 nm respectively

$Q_0 = A_2/A_1$, $Q_1 = aX_2 / aX_1$, $Q_2 = aY_2 / aY_1$.

aX_1 (15.47) and aX_2 (17.62) are the Absorptivity value of Ubidecarenone at 282.3 nm and 296.0nm respectively.

aY_1 (17.18) and aY_2 (15.94) are the Absorptivity value of Clomifene Citrate at 282.3 nm and 296.0nm respectively.

Analysis of Tablet formulation

For the assay of Ubidecarenone and Clomifene Citrate in combined tablet formulation (Ubiphene), twenty tablets were weighed and crushed in to fine powder. The quantity of powder equivalent to 24 mg of Ubidecarenone and 20 mg of Clomifene Citrate was transferred in to 50 ml volumetric flask containing 1 ml of Hexane and 20 ml of 25% v/v Ethanol in Methanol was added. The content of the flask was sonicated for 10 minutes and the volume was then made up to the mark with 25% v/v Ethanol in Methanol^{11, 12}. The solution was then filtered using Whatmann filter paper and the filtered solution was further diluted with the same solvent to get the concentration of 24 µg/ml of Ubidecarenone and 20 µg/ml of Clomifene Citrate. The concentration of Ubidecarenone and Clomifene Citrate present in the mixed sample solution was determined by applying the observed absorbance at the specified

wavelengths in the equations mentioned in Method-I and Method-II¹³.

RESULTS AND DISCUSSION

From the overlain spectrum was show in Figure No.1, the absorption maxima of Ubidecarenone and Clomifene Citrate were found to be 275.0 nm and 296.0 nm respectively. Both components having the same absorbance at 282.3 nm (Isoabsorptive point) and this wavelength were selected in Simultaneous equation method. The marketed formulation was analyzed as per the proposed methods and the percentage assay was found in the range of 100.75 – 101.10% and 98.96 – 102.24% for Ubidecarenone and Clomifene Citrate respectively show in Table No.1 and Figure No.1. The developed methods were validated in terms of linearity, accuracy, precision and reproducibility. The linearity was observed in the concentration range of 12-36 µg/ml for Ubidecarenone and 20-24 µg/ml for Clomifene Citrate. Standard calibration curves for Ubidecarenone and Clomifene Citrate with correlation coefficients values of 0.9995 and 0.9992 in Table No.2 and Figure No.2, 3 at all the selected wavelengths. The accuracy of the methods was checked by performing the recovery studies of the drugs at three different levels 80, 100, 120%. A known amount of Ubidecarenone and Clomifene Citrate was added to the pre-analyzed tablet formulation and the percentage recovery of the drug was calculated by the proposed methods. The percentage recovery of the drugs was ranges from 99.05 – 102.80% for Ubidecarenone and 98.50 – 101.46% for Clomifene citrate in Table No.3 and Figure No.4. The precision of the methods was investigated at three different levels (50%, 100% and 150%) by checking the Inter-day and intra- day variation of the results Table No.4 and Figure No.5 expressed as relative standard deviation (RSD). The % RSD of the results were found to be less than 2.0% indicates the good precision of the methods.

Table No.1: Results of Analysis of Tablet Formulations

S.No	Method	Label claim(mg/tab)		Amount found*(mg/tab)		Assay* (%)	
		UBI	CLO	UBI	CLO	UBI	CLO
1	I	30	25	30.22	24.26	100.75	101.10
2	II	30	25	30.67	23.75	102.24	98.96

*Mean of six determinations UBI- Ubidecarenone, CLO- Clomifene Citrate

Table No.2: Results of Linearity of the Methods

S.No	Methods	Simultaneous equation method		Q- Absorbance ratio method	
		UBI	CLO	UBI	CLO
1	Parameters	UBI	CLO	UBI	CLO
2	λ_{max}	275.0 nm	296.0 nm	275.0 nm	296.0 nm
3	Linearity	12-36 μ g/ml	10-30 μ g/ml	12-36 μ g/ml	10-30 μ g/ml
4	Slope	0.0603	0.0492	0.0597	0.0502
5	Intercept	-0.00030	0.00120	-0.00028	0.00114
6	LOD(μ g/ml)	3.24	2.70	3.58	2.98
7	LOQ(μ g/ml)	10.54	8.05	10.86	8.11
8	Correl. coefficient	0.9995	0.9994	0.9998	0.9992

UBI- Ubidecarenone, CLO- Clomifene Citrate

Table No.3: Results of Recovery Studies of Marketed Formulations

S.No	Level of Recovery	Drug	Amt of drug added in μ g/ml	Method I		Method II	
				% Recovery*	\pm RSD*	% Recovery*	\pm RSD*
1	80%	UBI	19.2	101.40	0.623	102.32	0.874
		CLO	16.0	99.20	0.234	100.56	0.376
2	100%	UBI	24.0	102.80	0.421	101.80	1.254
		CLO	20.0	98.93	0.092	98.50	0.605
3	120%	UBI	28.8	99.54	0.425	99.05	1.032
		CLO	24.0	100.60	0.540	101.46	0.578

*Mean of six determinations UBI- Ubidecarenone, CLO- Clomifene Citrate

Table No.4: Results of Repeatability Studies

S.No	Method	Drug	Amt of drug taken in μ g/ml	Inter-day		Intra-day	
				Amount found*	\pm SD*	Amount found*	\pm SD*
1	Method I	UBI	24.2	24.05	0.458	23.84	0.584
		CLO	19.8	19.94	0.784	19.78	0.267
2	Method II	UBI	23.9	23.52	0.384	23.69	1.142
		CLO	20.0	19.74	1.024	20.24	0.561

*Mean of six determinations UBI- Ubidecarenone, CLO- Clomifene Citrate

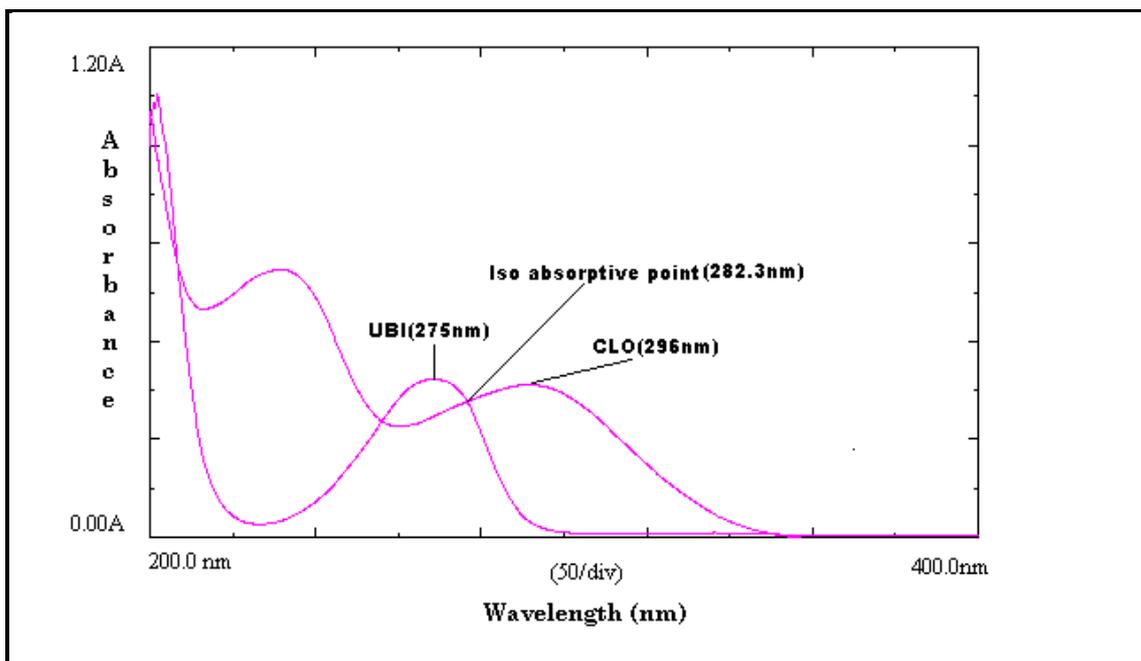


Figure No.1: Overlain spectrum of Ubidecarenone and Clomifene Citrate

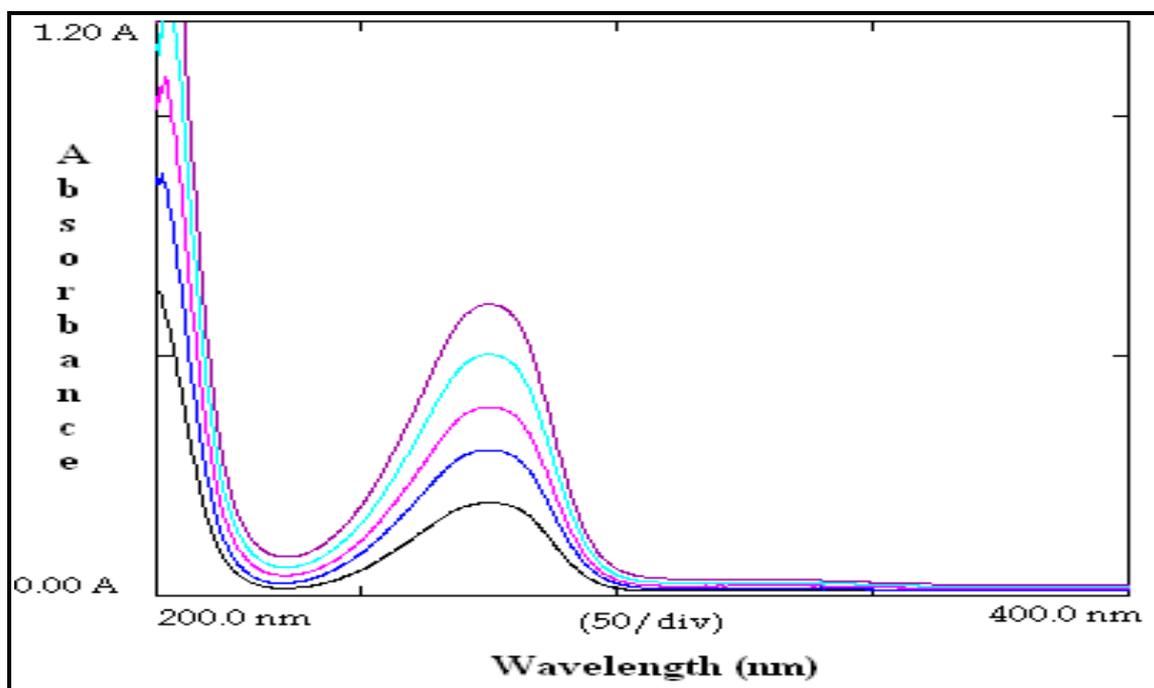


Figure No.2: Linearity spectrum of Ubidecarenone

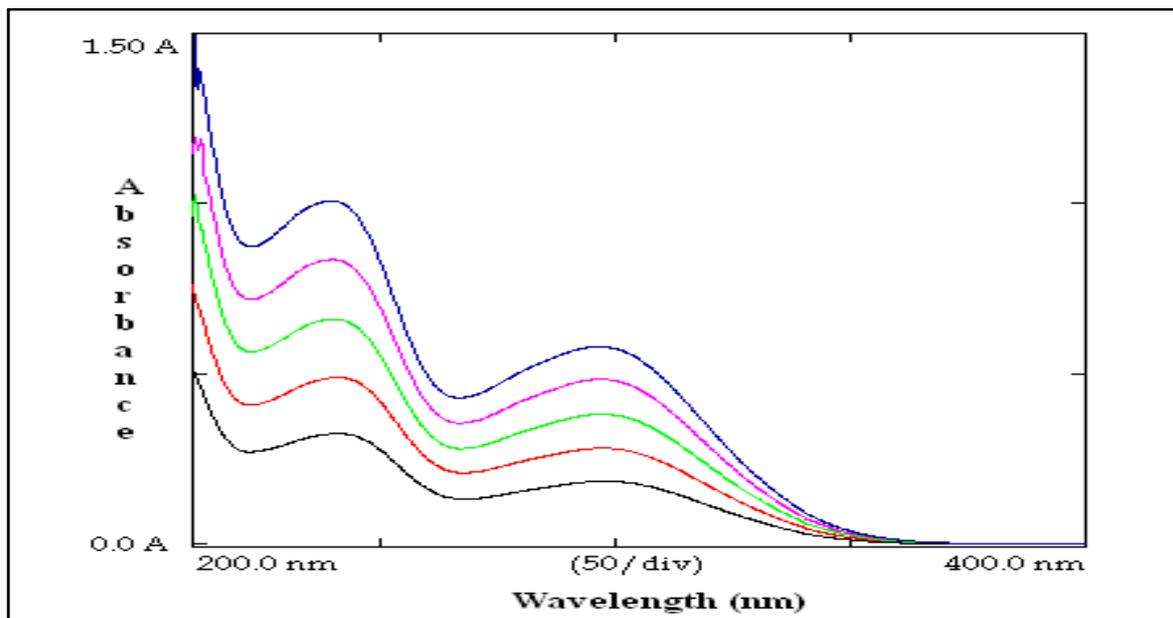


Figure No.3: Linearity spectrum of Clomifene Citrate

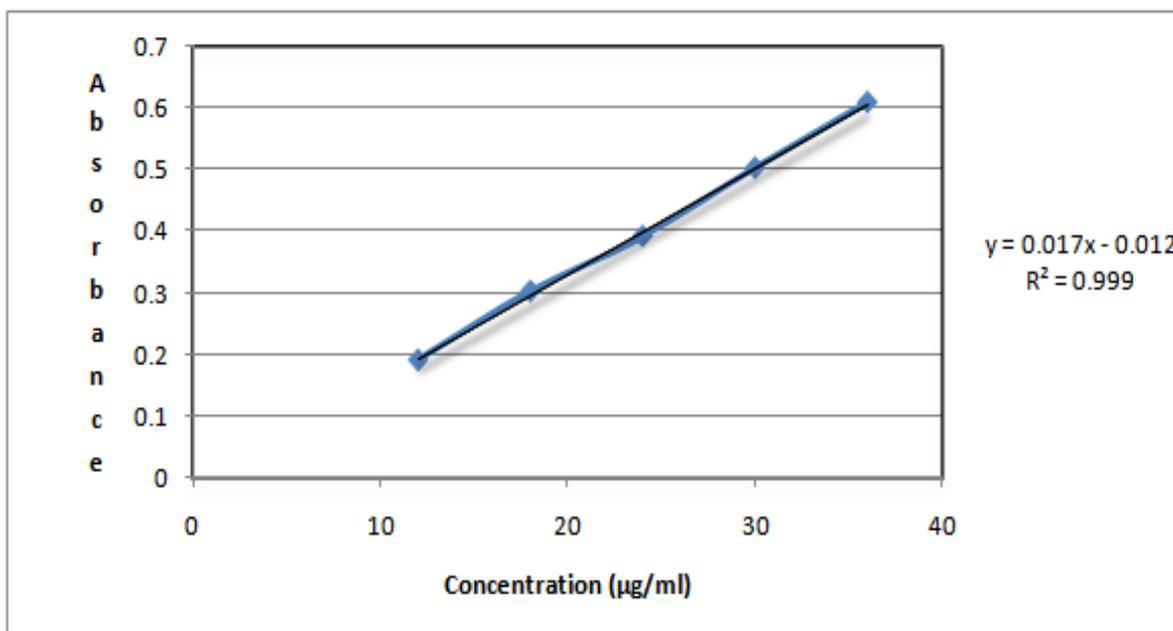


Figure No.4: Linearity graph of Ubidecarenone at 275nm

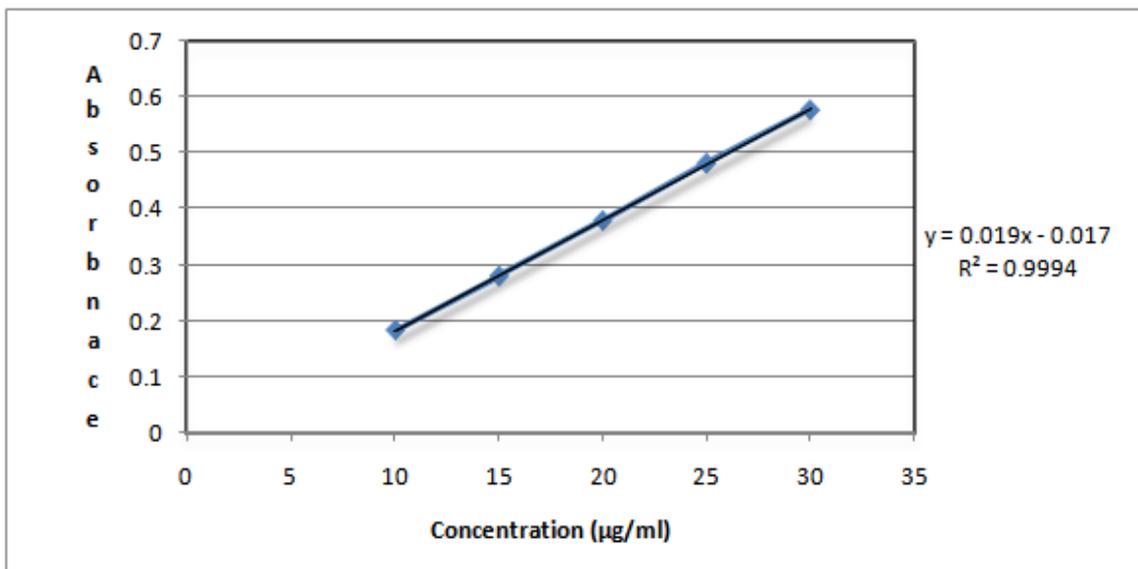


Figure No.5: Linearity graph of Clomifene Citrate at 296 nm

CONCLUSION

The proposed methods for the simultaneous estimation of Ubidecarenone and Clomifene Citrate were found to be simple, accurate, precise, rapid and economical and can be used for routine analysis of pharmaceutical formulations in quality control laboratories.

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