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HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF UBIDECARENONE AND CLOMIFENE CITRATE IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

A validated reverse phase HPLC method has been developed for the simultaneous estimation of Ubidecarenone and Clomifene Citrate in Pharmaceutical dosage forms. The chromatographic separation was carried out on Phenomenex C_8 (250 X 4.6 mm, 5 µm) column and Methanol: Ethanol: Hexane in the ratio of 80:10:10% v/v was used as mobile phase at the flow rate of 1.5 ml/min with PDA detection at 275 nm. The retention time of Ubidecarenone and Clomifene Citrate were found to be 2.37 min and 9.72 min respectively. Linearity of both drugs were found to be in the concentration range of 15-45 µg/ml for Ubidecarenone and 12.5- 37.5 µg/ml for Clomifene Citrate. The developed HPLC method was validated by determining its sensitivity, selectivity, linearity, accuracy and precision. The accuracy of the method was assessed by percentage recovery studies at three different levels at 50%, 100% and 150% of its working concentration. The percentage recovery of both drugs in the developed method was found to be in the ranges of from 99.1% - 101.1%, that indicates the good accuracy of the method. This developed method can be used for the routine analysis for the estimation of Ubidecarenone and Clomifene Citrate in bulk and Pharmaceutical formulations.

KEY WORDS

Clomifene, Citrate, RP-HPLC Method and Ubidecarenone.

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INTRODUCTION

Ubidecarenone is chemically 2- [(2E, 6E, 10E, 14E, 18E, 22E) -3, 7, 11, 15, 19, 23, 27, 31, 35, 39- decamethyl 2, 6, 10, 14, 18, 22, 26, 30, 34, 38- tetracontadecaenyl]-5, 6- dimethoxy, 3 methyl benzene. It is used as a dietary1, 4 dione, which is

also used as 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 methods. 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-, 2diphenylvinyl) 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 product. These two methods are mentioned in Japanese Pharmacopoeia 15th edition 2009. Literature review revealed that several methods have been reported for the quantification of Ubidecarenone and Clomifene Citrate individually[1-2]. However there is no HPLC methods have been reported for the simultaneous estimation of Ubidecarenone and Clomifene Citrate in Pharmaceutical formulations. The present work describes a new, simple, rapid, accurate and precise RP-HPLC method developed and validated for the estimation of Ubidecarenone and Clomifene Citrate simultaneously.

MATERIAL AND METHOD

Chromatographic separation was carried out on Shimadzu Prominence liquid chromatographic system equipped with quaternary pump, PDA detector and auto injector. LC solution software (Version 1.23) was used for the entire processing and data collection. All chemicals used were analytical grade and the solvents which are used in the mobile phase were HPLC grade. Reference standard and combined tablet formulation of Ubidecarenone and Clomifene Citrate were obtained as a gift sample from M/s. Fourrts (India) Laboratories, Chennai.

Preparation of standard solution

Standard solution of Ubidecarenone and Clomifene Citrate was prepared by dissolving 30 mg of Ubidecarenone WS in a 100 ml volumetric flask containing 5 ml of Diethyl ether and 25 mg of Clomifene Citrate WS, 70 ml of methanol were added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with methanol. The resulting solution was further diluted with methanol to get the concentration of 30 µg/ml of Ubidecarenone and 25 µg/ml of Clomifene Citrate[3-4].

Preparation of sample solution

Accurately weighed quantity of sample equivalent to 30 mg of Ubidecarenone and 25 mg of Clomifene Citrate was taken in a 100 ml volumetric flask and 5 ml of Diethyl ether, 70 ml of methanol were added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with methanol. The resulting solution was filtered through whatman filter paper and 5 ml of the filtrate was diluted to 50 ml with methanol.

Method development and validation[5-10]

The RP HPLC procedure was optimized with a view to develop an effective method for the simultaneous estimation of Ubidecarenone and Clomifene Citrate in tablet dosage forms. Preliminary tests were performed in order to select the adequate and optimum chromatographic condition. A Phenomenex C_8 column was used as a stationary phase and the separation was achieved by using mobile phase consisting of Methanol: Ethanol: Hexane in the ratio of 80:10:10% v/v in isocratic mode. Chromatogram of standard solution containing Ubidecarenone and Clomifene Citrate is shown in Figure No.1. The developed HPLC method for the simultaneous estimation Ubidecarenone and Clomifene Citrate was validated as per the ICH guideline in terms of specificity, linearity, accuracy, precision, ruggedness and robustness, limit of detection and limit of quantification.

Specificity

The specificity of the method was determined by spiking the solution of placebo with the working

standard solution containing Ubidecarenone and Clomifene Citrate and this solution was analyzed as per the method described. The recorded chromatogram was compared with chromatogram of standard solution containing Ubidecarenone and Clomifene Citrate to check the interference of the placebo with the response produced by the Ubidecarenone and Clomifene Citrate.

System suitability

The system suitability of the method was determined by five replicate analysis of the standard solution containing Ubidecarenone and Clomifene Citrate to check the reproducibility of the chromatographic system. In this method the reproducibility of peak area, retention time, theoretical plate and tailing factor of the peaks of Ubidecarenone and Clomifene Citrate were checked. The overlain chromatogram of system suitability test is shown in Figure No.2.

Linearity

The linearity of the method was assessed by analyzing the standard solution containing Ubidecarenone and Clomifene Citrate at 5 different levels 50%, 80%, 100%, 120% and 150% of its working concentration. The calibration curve of peak area (vs) concentration was plotted and correlation coefficient and regression line equation for both drugs were determined. The calibration curve of Ubidecarenone and Clomifene Citrate is shown in Figure No.3 and Figure No.4 respectively.

Accuracy

Accuracy of the method was assessed by analyzing the solutions containing Ubidecarenone and Clomifene Citrate at three different levels 50%, 100% and 150% of its working concentration. Standard solutions were spiked with placebo and the percentage recovery of the drugs from the placebo was calculated. The overlain chromatogram of accuracy test is shown in Figure No.5.

Precision

Intra-day precision was determined by carrying out three independent assays of both drugs at three different time points on the same day. Inter-day precision of the method was determined by analyzing of both drugs at three different time points on different days. The % RSD of the obtained results was calculated.

Ruggedness and robustness

The ruggedness of the method was ascertained by carrying out the assay of the sample on different instrument by different analyst using different column of similar types. The chromatogram which is recorded for ruggedness studies is shown in Fig No.6. Robustness of the method was determined by analyzing the sample by deliberately changed chromatographic conditions such as change in mobile phase composition (±2 ml), flow rate (0.1 ml/min) and detection wavelength (±2 nm).

LOD and LOQ

The limit of detection and limit of quantification of Ubidecarenone and Clomifene Citrate were calculated by using standard deviation of the responses and the slope of the calibration curve of Ubidecarenone and Clomifene Citrate. LOD and LOQ were estimated by using the following formula,

$$LOD = (3.3 \times \sigma) / S$$

$$LOQ = (10 \times \sigma) / S$$

Where σ is the standard deviation of the response

S is the slope of the calibration curve.

Analysis of Ubidecarenone and Clomifene Citrate in Tablet formulation

For the assay of Ubidecarenone and Clomifene Citrate in tablet formulations, twenty tablets were weighed and the average weight of the tablets was calculated. The weighed tablets were crushed in to fine powder. A quantity of powder equivalent to 30 mg of Ubidecarenone and 25 mg of Clomifene Citrate was transferred in to 100 ml volumetric flask containing 5 ml of Diethyl ether and 70 ml of Methanol was added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with methanol. This solution was filtered through the Whatman filter paper and 5ml of the filtrate was diluted to 50ml with methanol. Form the resulting solution 20 µl was injected in to the column and response was recorded under the same chromatographic

conditions. Six such samples were prepared and analyzed in the same manner. The amount of Ubidecarenone and Clomifene Citrate present in the sample was determined by comparing the mean peak area of sample with that of standard.

RESULTS AND DISCUSSION

A simple, accurate and precise RP HPLC method was developed for the simultaneous estimation of Ubidecarenone and Clomifene Citrate in Pharmaceutical dosage forms. All the results were summarized in Table No.1. Specificity of the method was tested by comparing the response of blank, standard and placebo mixed sample solution. No interference of placebo was detected at the retention time of Ubidecarenone and Clomifene Citrate. The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. The results of the system suitability test were summarized in Table No.2. Linearity of the method was evaluated at 5 different concentration levels 15-45 µg/ml and 12.5-37.5 µg/ml for Ubidecarenone and Clomifene Citrate respectively. Both the drugs were found to give linear detector response in the concentration under study with correlation coefficient of 0.9994 and 0.9991 for Ubidecarenone and Clomifene Citrate respectively. Accuracy of the method was determined by recovery test. The percentage recovery was found to be in the range of 99.10% - 100.82% for Ubidecarenone and 99.42 % - 101.3% for Clomifene Citrate (Table No.3). All results indicate that the method is highly accurate. This method was validated for its inter-day and intra-day precision. The results obtained were within the acceptable limit. The ruggedness and robustness of the method were determined and the % RSD of the results were found to be less than 2.0%, which demonstrate that the developed method is rugged and robust. Detection limit for Ubidecarenone and Clomifene Citrate was 0.37µg/ml and 0.66 µg/ml and quantification limit was 0.12 µg/ml and 0.21 µg/ml, which suggest that a nanogram level of both drugs can be estimated accurately. All the results of validation parameters are summarized in the Table 3. The solvents which had been used in the mobile phase were cost effective than the solvents used in the other HPLC methods which are reported in the literatures.

Table No.1: Results of Validation of the developed HPLC Method

S.No	Parameters*	Ubidecarenone	Clomifene Citrate
1	Linearity (µg/ml)	15-45	12-36
2	Correl. coefficient	0.9995	0.9999
3	% Recovery	99.10% to 99.65%	100.64% to 101.10%
4	Inter- day precision (% RSD)	0.478	0.564
5	Intra- day precision (% RSD)	0.284	0.687
6	Robustness (%RSD)	0.351	1.054
7	Ruggedness (%RSD)	0.128	0.282
8	LOD(µg/ml)	0.12	0.21
9	LOQ(µg/ml)	0.37	0.66

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

Table No.2: Results of System Suitability Parameters for the analysis of Ubidecarenone and Clomifene Citrate

S.No	Parameters*	Ubidecarenone	Clomifene Citrate
1.	Peak area	397390	342541
2.	Theoretical Plates	3566.40	2275.77
3.	Tailing Factor	1.16	1.21
4.	Retention time (minutes)	2.37	9.72
5.	Resolution	0.0	16.63
6.	% RSD of Peak area	0.245	0.312

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

Table No.3: Results of Recovery Studies of Marketed Formulation (Ubiphene)

S.No	Level of Recovery	Drug	Amount of drug added in µg/ml	% Recovery*	± RSD*
1	50%	UBI	15.0	99.6	0.384
		CLO	12.5	100.6	0.412
2	100%	UBI	30.0	99.1	0.845
		CLO	25.0	101.1	0.749
3	150%	UBI	45.0	99.5	0.471
		CLO	37.5	100.8	0.711

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

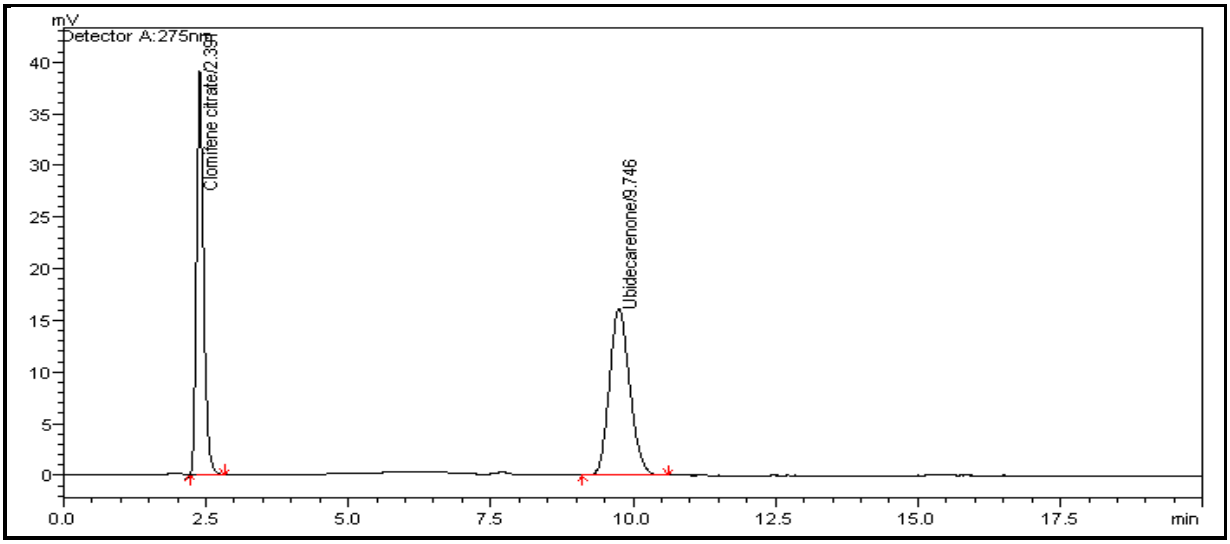

Figure No.1: Typical HPLC Chromatogram of Ubidecarenone (30µg/ml) and Clomifene Citrate (25µg/ml)

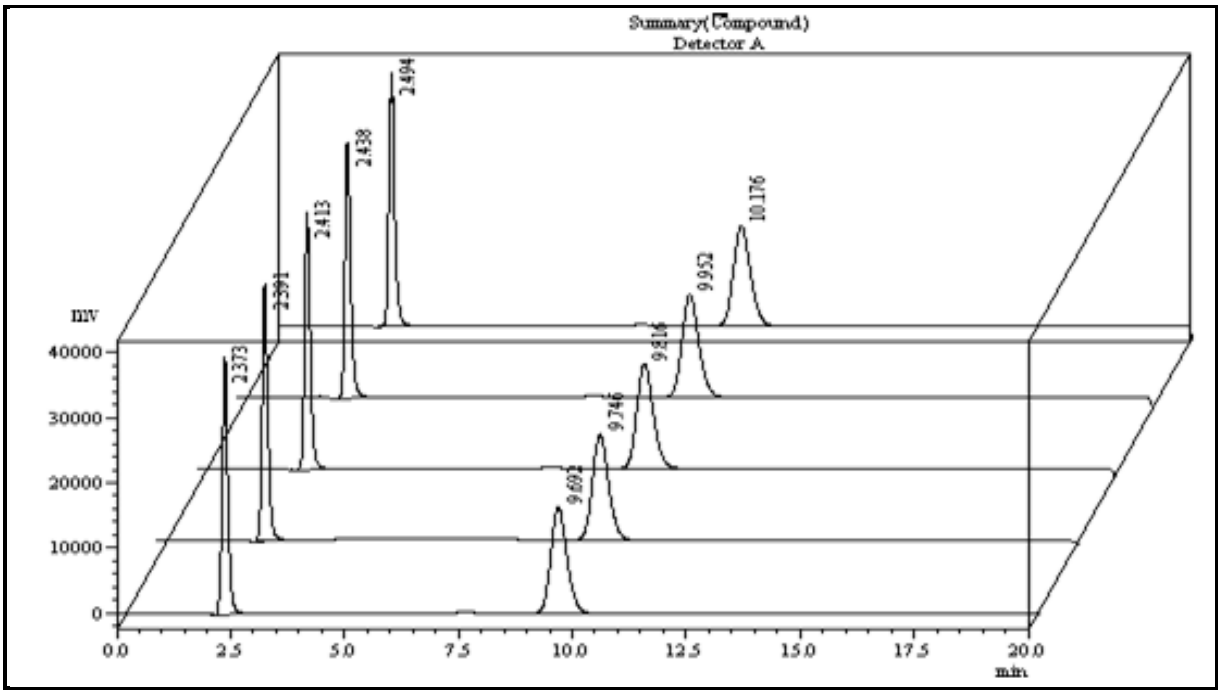

Figure No.2: Overlain Chromatogram of Ubidecarenone and Clomifene Citrate for System suitability test

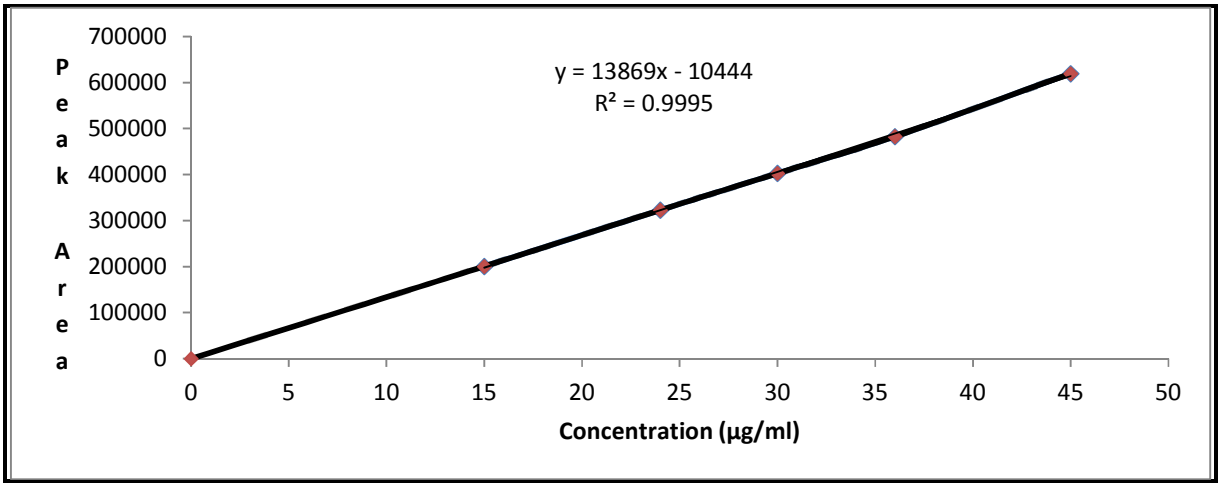

Figure No.3: Calibration curve of Ubidecarenone by HPLC

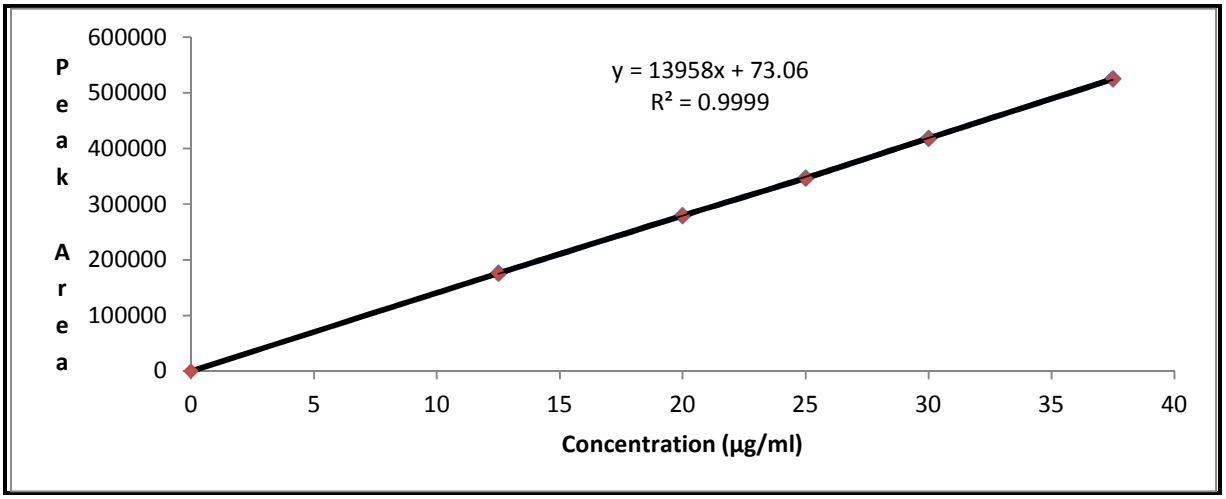

Figure No.4: Calibration curve of Clomifene Citrate by HPLC

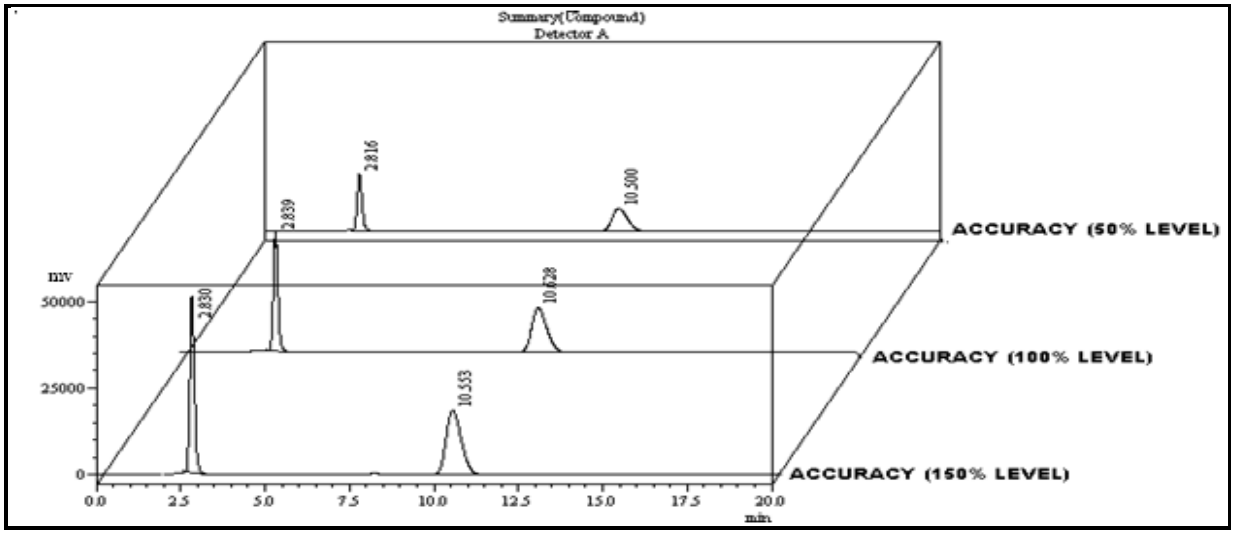

Figure No.5: Overlain Chromatogram of Ubidecarenone and Clomifene Citrate for Accuracy test at three different levels

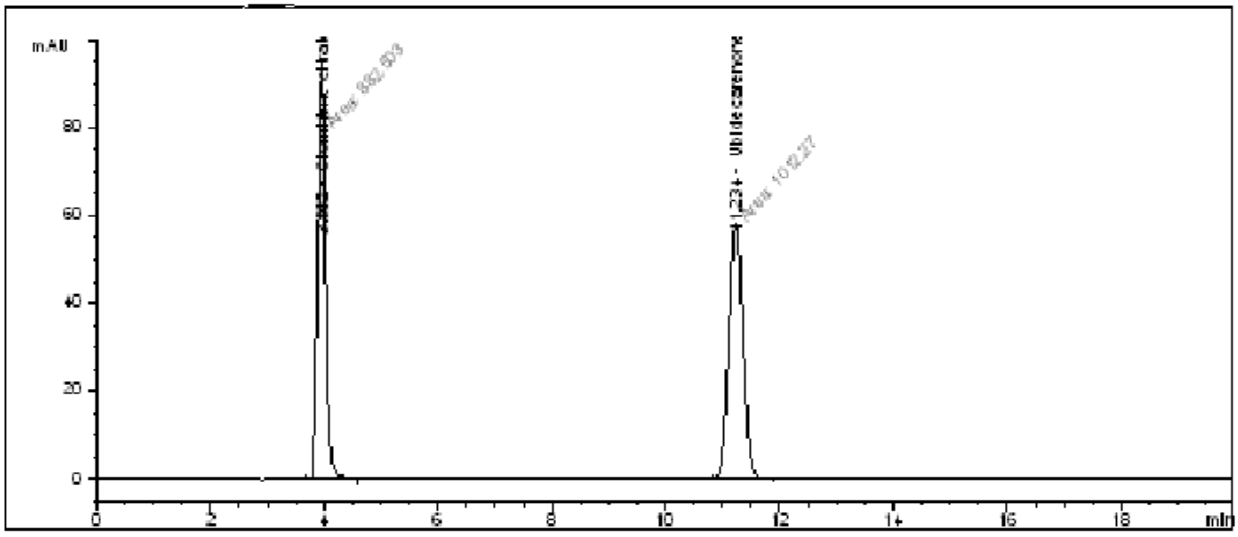

Figure No.6: Chromatogram of Ubidecarenone and Clomifene Citrate for Ruggedness test

CONCLUSION

The developed RP HPLC method for the simultaneous estimation of Ubidecarenone and Clomifene Citrate offers simplicity, selectivity, precision and accuracy. All the results of validation parameters are summarized in the Table No. 3. The solvents which had been used in the mobile phase

The method gives good resolution between the compounds with a short analysis time. So the developed method can be used for the routine analysis of Ubidecarenone and Clomifene Citrate in bulk and Pharmaceutical formulations.

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for providing the necessary facilities to carry out this work.

were cost effective than the solvents used in the other HPLC methods which are reported in the literatures.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. The Japanese Pharmacopoeia. *The Society of Japanese Pharmacopoeia,* 2009, 2109.
2. The European Pharmacopoeia. *Strasbourg, France: EDQM, Council of Europe,* Edition-5, 2005, 2157.
3. Sethi P D. HPLC Quantitative Analysis of Pharmaceutical formulations, *CBS Publisher and distributor, New Delhi,* 1996.
4. International Conference on Harmonization. Draft Guideline on Validation of analytical procedures: Methodology, *Federal Register*, Nov.1996, 1-8.
5. Saravanamuthukumar M, Palanivelu M, Anadarajagopal K and Sridharan D. Simultaneous estimation and Validation of Atorvastatin Calcium and Ubidecarenone in combined Tablet Dosage forms by RP-HPLC Method, *Int J Pharm Pharm Sci*, 2(2), 2010, 36-38.
6. Zhou T Y, Sun H D Zhang L and Zang Q. Determination of total coenzymes in plasma, *Chinese Pharm J*, 37(3), 2002, 189-192.
7. Sreekanth N, Babu Rao CH and Mukkanti K. RP-HPLC Method Development and Validation of Ropinirole Hydrochloride in Bulk and Pharmaceutical Dosage Forms, *Int J Pharm Pharm Sci*, 1(1), 2009, 186-192.
8. Karthik A, Subramanian G, Ranjith Kumar A and Udupa N. Simultaneous Estimation of Paracetamol and Domperidone in Tablets by Reverse Phase HPLC Method, *Indian J. Pharm. Sci*, 69(1), 2007, 142-144.
9. Sivakumar T, Manavalan R and Valliappan K. Stability indicating HPLC Method for Simultaneous Estimation of Pantoprazole and Domperidone from their Combination Drug Product, *Acta Chromatophia*, 18(1), 2007, 130-142.
10. Sunil J, Sanjithnath M and Sambamoorthy U. HPLC Method development and Validation for Simultaneous estimation of Artemether and Lumifantrene in Pharmaceutical Dosage Forms, *Int J Pharm Pharm Sci*, 2(4), 2010, 93-96.

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