

**Original Research Article****Validated Chromatographic method for Estimation of CoQ10 in Bulk Drug and Self-Nanoemulsifying Formulation**

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ARTICLE INFO:**Article history:**

Received: 13 January, 2016

Received in revised form:

20 January, 2016

Accepted: 25 January, 2016

Available online: 29 February, 2016

Keywords:

CoQ10

Method validation

Precision

Bulk drug

RP-HPLC

ABSTRACT

A simple, precise and sensitive RP-HPLC method was developed and validated for the routine analysis of CoQ10 in bulk drug and self-nanoemulsifying formulation. The analyte was quantified using column C₁₈ which configurationally has a binary pump system, a UV-detector and an injector. The mobile phase consisted of methanol and *n*-hexane in the ratio of (80:20 % v/v) flowing gradiently at a flow rate of 1 ml/min. The wavelength monitored at 275 nm for estimation of drug. The previously weigh quantity of drug was transferred in mobile phase, filtered through membrane of pore size 0.45 micron and injected into column. The designed parameter was validated for specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) and robustness. The graph was linear over the concentration range of 0.010-10 µg/ml with a correlation coefficient $R^2 = 0.999$. The LOD and LOQ value of CoQ10 was determined 0.0032 and 0.01 µg/ml. The method was successfully validated as per ICH guidelines and found to be simple, precise and accurate for the estimation of CoQ10. It has great applicability in assessing CoQ10 in self-nanoemulsifying formulation developed in the lab and other marketed formulation.

1. Introduction

Naturally CoQ10 is a vitamin like substance which is freely soluble in fat. It acts as antioxidant inside the cell and also as electron carrier in respiratory chain of mitochondria[1,2]. Due to its structural geometry and empirical formula weight, the aqueous solubility is very low resulting erratic bioavailability and slow absorption[3]. Therapeutically it was proved to be helpful in cardiac disorder[4], neuroprotective, and exerts anti-inflammatory property by diminishing secretion of pro-inflammatory cytokines[5]. CoQ10 combine with apoenzyme that forms active enzyme which involve in breakdown of protein into amino acids and helps in maintaining homeostasis[6]. Based on the literature review on analytical procedure of CoQ10 few papers reported yet[7- 9]. Literature also reported the estimation of CoQ10 by HPLC-MS but that require sophisticated instrument, technical personnel, more economic and not feasible to carry out routinely. HPLC with UV-detector is commonly used for quantification[10].

2. Materials and Methods**2.1. Materials**

CoQ10 was a gift sample from from Sami Lab (Bangalore, India). The reagent for buffer preparation potassium dihydrogen phosphate, sodium hydroxide, HPLC grade water, methanol and *n*-hexane were purchased from Central Drug House (CDH) (New Delhi, India). The preparation, reagent

and solution used were filtered through a 0.45 µ membrane filter and degassed before application. The injection samples were further filtered through Durapore membrane of size 0.22 µ. Fresh stock solution was prepared on every day, all chemicals and reagent used were of HPLC grade and used as received.

2.2. Apparatus

The HPLC system was used (SHIMADZU, Japan) consisting of a UV detector, a binary pump system, and an injector. The separation of analyte was achieved in Colligen[®]100 column C₁₈ of pore size, length and diameter (5 µm, 250 × 4.6 mm) and elution was performed gradiently. The mobile phase composed of methanol and *n*-hexane in ratio of (80:20 % v/v). The observation was made at set wavelength of 275 nm and flow rate of mobile phase was maintained 1 ml/min.

3. Method**3.1. Preparation of stock and standard solution**

Stock solution of CoQ10 was prepared by dissolving 10 mg of CoQ10 in 100 ml of methanol in a 100 ml volumetric flask to obtain the concentration of 100 µg/ml. A series of standard solutions at concentrations of 0.01, 2.0, 4.0, 6.0, 8.0 and 10 µg/ml were prepared by dilution of stock solution to obtain different calibration standard and stored at 4 °C as per study protocol[11].

3.2. Preparation of calibration curve

The calibration curve was made from standard concentration range 0.01 to 10 µg/ml. The samples were taken from above calibration standard solution and 100 µl of each solution were injected into the chromatographic column. The calibration curve was established by plotting peak area (y) of CoQ10 to the concentration of CoQ10 (x) as shown in Figure No. 1.

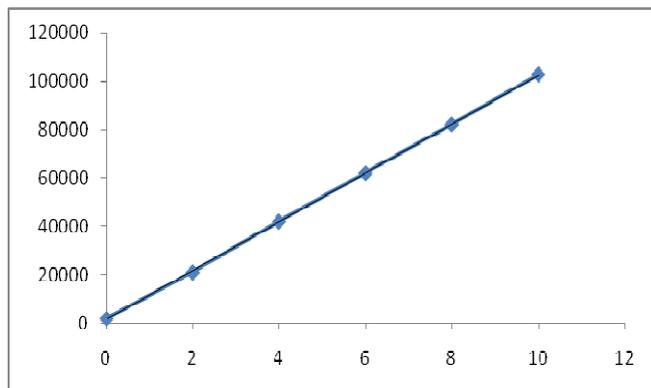


Figure No. 1: Calibration plot of CoQ10 by HPLC

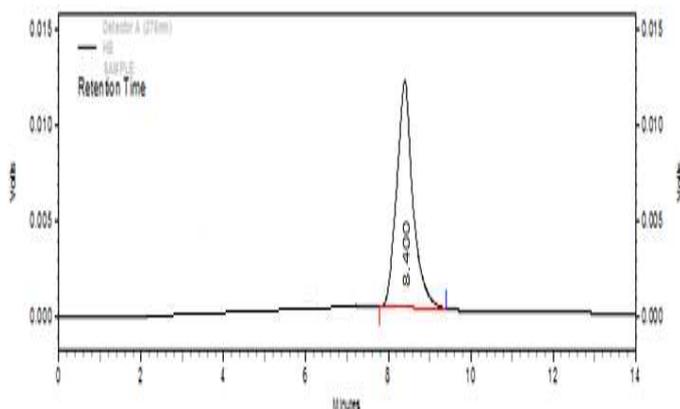


Figure No. 2: A typical HPLC chromatogram of CoQ10 reference sample

4. Analytical method validation

A simple, sensitive, reliable, and cost-effective, HPLC method, validated in accordance with International Conference on Harmonization (ICH) guidelines for determination of CoQ10 includes following parameters[12,13].

4.1. Linearity

The linearity was predicted over the concentration range of 0.01 to 10 µg/ml. This study signifies that method is able to analyze the sample solution in concentration range where analyte response is linearly proportional to concentration.

4.2. Assay precision and accuracy

Precision is the variation of repeated result while, accuracy is closeness to a true value. The intra- day variation of the assay were determined by replicate analysis ($n = 3$) of calibration samples of CoQ10 at concentrations within the range of

calibration curve (0.010-10 µg /ml) in a single analytical run on the same day. In other hand, the inter-day precision was evaluated by analysis of the samples at three different concentration (0.010, 6, 10 µg /ml) in different day ($n = 3$). Precision was measured by percentage relative standard deviation (% RSD) and calculated as; % RSD = (Standard Deviation /Mean) x100[14]. The accuracy was evaluated by the deviation or bias (%) of the observed concentration from the actual concentration. The accuracy of the proposed analytical method was evaluated by adding three known concentrations of drug equivalent to 50, 100 and 150 % to the original concentration and determining the recovery of added drug. The experiment was performed in triplicate ($n=3$).

4.3. LOD and LOQ

The limit of detection (LOD) is the smallest amount of a sample that can be differentiated from background noise but not quantified. The LOQ was identified as the lowest drug concentration of the standard curve that could be quantified within the range of accuracy and precision.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using standard deviation (SD) of the y-intercept and slope (S) of the calibration curve as per the given formulae:

$$\text{LOD}=3.3*\sigma/S \text{ and } \text{LOQ}=10*\sigma/S$$

The data obtained in this experiment was assessed by using statistical GraphPad Prism 4.0 (GraphPad Software, Inc., USA). Experiment was performed in triplicate ($n=3$).

4.4. Specificity or selectivity

The specificity is referred to produce a response for single analyte. The method is considered to be selective a single response for specific analyte in presence of other interferences.

4.5. Robustness

The robustness of the method was determined by varying the different chromatographic parameters like mobile phase composition and flow rate to determine their influence on the quantitative analysis.

5. Assay of CoQ10 in bulk drug and self-nanoemulsifying formulation

Accurately weigh quantity of self-nanoemulsifying formulation (~10 mg) transferred to volumetric flask containing methanol to extract the CoQ10 in the solvent. The solution was filtered through 0.45 µm membrane filter. The solution was further diluted with mobile phase to get 4 µg/ml and analyzed by HPLC. The mean values of peak area were calculated and the drug content in the formulation was quantified using calibration curve.

6. Result and discussion

The calibration curve was linear in the concentration range of 0.01-10 µg/ml. The linearity were established by given equation $y = mx + c$, where y represents the peak area of CoQ10, x represents the concentration of CoQ10, m is the slope of the plot, and c is the intercept, the equation obtained

from six-point calibration plot was $y = 10096x + 1443$ with regression coefficient of $R^2 = 0.999$ (Figure No. 1). The intraday precision of the assay was determined by analysis of three different concentrations (0.01, 6 and 10 $\mu\text{g/ml}$) on the same day. For determination of inter-day precision, the samples were analyzed on different days. Each sample were injected in triplicate ($n=3$). The average % RSD values for intra and inter-day precision was 0.64 % and 0.79 % (< 1 %). The accuracy of the assay method was determined and % RSD reported 0.48 % (< 1 %). The mean recovery of CoQ10 at concentration of 0.01, 2, and 4 $\mu\text{g/ml}$ was varies from 99.20 to 100.73 %. The LOD and LOQ value of CoQ10 was determined 0.0032 and 0.01 $\mu\text{g/ml}$. The term robustness means for small deliberate change in chromatographic condition such as change in flow rate and mobile phase composition for the estimation of CoQ10. It was determined by introducing small changes in the flow rate (0.8 and 1.2 mL/min) and composition of the mobile phase (± 2 %). The total run time of analyte was 14 min and retention time of CoQ10 was obtained at 8.4 min of reference sample (Figure No. 2). There was no significant change in the retention time of drug by changing the composition of the mobile phase and flow rate ($p < 0.05$). The % RSD value was (< 1 %) indicated robustness of the assay method.

7. Assay of CoQ10 in bulk drug and self-nanoemulsifying formulation

The application of assay method was estimated by quantifying CoQ10 in developed formulation inside the laboratory that shows high value of percentage recovery and % RSD was ($< 1\%$). The presence of formulating excipients showed no interference with the peak of analyte which further confirmed high selectivity of assay method. The change in retention time of CoQ10 in bulk sample and nanoformulation was insignificant ($p < 0.05$).

8. Conclusion

The proposed method was simple, precise, accurate, and reproducible for determination of CoQ10 in bulk samples and pharmaceutical dosage form. The high accuracy and good precision was established by statistical analysis. The low value of % RSD indicates that developed method has great applicability in routine analysis of CoQ10.

Acknowledgements

Md. Habban Akhter has grateful to Central Instrumentation Facility (CIF), Jamia Hamdard for providing facility of instrument reverse phase high performance chromatography (RP-HPLC) and Dr. Javed Ali, Senior Asst. Professor (Jamia Hamdard) for valuable discussion.

Source of support: Nil

Conflict of interest: None Declared

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Source of support: Nil, Conflict of interest: None Declared

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