Densitometric HPTLC method for qualitative, quantitative analysis and stability study of coenzyme Q10 in Pharmaceutical formulations utilizing normal and reversed-phase silica gel plates

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Abstract: Two simple, preciseand stability-indicating densitometric HPTLC method were developed and validated for qualitative and quantitative analysis of Coenzyme Q10in pharmaceutical formulations using normal-phase (Method I) and reversed phase (Method II) silica gel TLC plates. Both methods were developed and validated with 10×20cm glass-backed plates coated with 0.2mm layers of either silica gel 60 F_{254} (E-Merck, Germany) using hexane-ethyl acetate (8.5:1.5v/v) as developing system (Method I) or RP-18 silica gel 60 F_{254} (E-Merck, Germany) using methanol-acetone (4:6v/v) as mobile phase (Method II). Both analyses were scanned with a densitometer at 282 nm. Linearity was found in the ranges 50-800ng/spot (r^2=0.9989) and 50-800ng/spot (r^2=0.9987) for Method I and Method II respectively. Stability of Coenzyme Q10 was explored by the two methods using acid, base, hydrogen peroxide, temperature and different solvents. Due to the efficiency of the method in separating Coenzyme Q10 from other ingredients including its degradation products, it can be applied for quality control, standardization of different pharmaceutical formulations and stability study.

Keywords: Coenzyme Q10; HPTLC densitometry; ICH guidelines; qualitative; quantitative; stability.

INTRODUCTION

Ubiquinone (coenzyme Q10, CoQ10, ubidecarenone, coenzyme Q) is an oil-soluble, vitamin-like substance present in most eukaryotic cells, primarily in the mitochondria. Coenzyme Q are family of compounds differ in the number of isoprenoid subunits of the side-chains. CoQ10 is the major form of Coenzyme Q in human mitochondria. CoQ10 is a vital component in the electron transport chain essential for aerobic cellular respiration, generating chemical energy in the form of ATP (Ernster and Dallner, 1995; Leonard *et al*., 2000). Logically, the amount of CoQ10 is higher in the organs with the highest energy demands such as the heart, liver and kidney (Okamoto *et al*., 1989; Aberg *et al*., 1992; Shindo *et al*., 1994). To perform its role as antioxidant and component in electron transport chain CoQ10 can exist as reduced or oxidized form. The reduced form of CoQ10 molecule can easily donate one or both electrons and act as an antioxidant (Michael and Schachter). One of the most important CoQ10 function is the inhibition of lipid per oxidation through lipid peroxyl radicals (LOO) inhibition. Coenzyme Q10 is essential to keep the cardiovascular system in healthy condition. Heart failure is usually accompanied by deficiency of CoQ10. The concentration of CoQ10 in plasma is considered as marker of mortality in chronic heart failure and long-term prognosis of chronic heart failure (Molyneux *et al*., 2008). Coenzyme Q10 supplements can help some people suffering from migraine headaches in a dose from 150 to 300 mg/day (Sándor *et al*., 2005). The value of coenzyme Q10 in the treatment of cancer, and as relief from cancer treatment side-effects have been explored (Sakano *et al*., 2006, Clinical Trials. gov NCT00096356, 2008; Clinical Trials. gov NCT00976131, 2013). Consumption of coenzyme Q10 resulted in reduction in systolic blood pressure by up to 17 mm Hg and diastolic blood pressure by up to 10 mm Hg without significant side-effects (Rosenfeldt *et al*., 2007).

Studies have also shown that the level of CoQ10 is less in diseased gum tissue compared with healthy gum tissue (Littarru *et al*., 1971; Nakamura *et al*., 1974). Oral administration of CoQ10 resulted in improving the gingival health, immune response in gum tissues and reversing the diseased gum conditions (McRee *et al*., 1993; Folkers *et al*., 1991; Wilkinson *et al*., 1976).

Topical application of CoQ10 on gum tissues improves periodontitis and gingivitis conditions (Hanioka *et al*., 1994). Animal experiments indicated that coenzyme Q10 taken as dietary supplement help in reduction of radiation damage to the animals' blood (Koryagin *et al*., 2002). Meat and fish are the richest source of dietary CoQ10. More than 50 mg/kg can be found in beef, pork and chicken heart and liver. Dairy products are inferior in their CoQ10 contents compared to animal tissues. Vegetable oils are rich in CoQ10. Within vegetables, parsley and perilla are the richest CoQ10 sources. Broccoli, grape and cauliflower are modest sources of CoQ10. Most fruit and

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berries are poor sources of CoQ10, with the exception of avocado with a relatively high CoQ10 content (Pravst *et al*., 2010).

Several HPLC methods were developed for the estimation of CoQ10 in Human plasma, raw materials and dietary supplements (Tang *et al*., 2001; Lunetta and Roman, 2008; Graves *et al*., 1998; Surasi *et al*., 1984). Analysis of an inclusion complex of CoQ10 with *β*-cyclodextrin was performed by TLC (Prosek *et al*., US Patent 6984308). A sensitive and selective analysis of CoQ10 in human serum was also achieved by negative APCI LC-MS (Hansen *et al*., 2004). CoQ10 was analyzed in Pharmaceutical formulations using FT-IR spectrophotometric method (Bunaciu *et al*., 2007). CoQ10 stability in pediatric liquid oral dosage formulations and its bioequivalence studies of two marketed formulations of coenzyme Q10 in beagle dogs has been reported (Estévez *et al*., 2012; Kommuru *et al*., 1999).

To the best of our knowledge, no reports on densitometric HPTLC analytical methods for the analysis of Coenzyme Q10in pharmaceutical formulation utilizing normal and reversed-phase silica gel plates have been mentioned in the literature. The objective of this study was, therefore, to develop a simple, accurate, selective, precise, sensitive, robust, and stability-indicating densitometric HPTLC method of CoQ10in pharmaceutical formulation utilizing both normal and reverse phase silica gel plates.

MATERIALS AND METHODS

Standard and materials

Standard CoQ10 was purchased from Sigma Aldrich. Pharmaceutical formulations were purchased from local market in Riyadh, Kingdom of Saudi Arabia and Alexandria, Egypt. All the solvents were of HPLC grade and other chemicals used were of analytical reagent (AR) grade.

Accurately weighed 10 mg of standard CoQ10 was dissolved in MeOH in a 100mL volumetric flask to gives concentration of 100µg/mL. This solution was used as a reference solution (stock solution) for CoQ10.

Two capsules and one-tablet pharmaceutical formulations were used for CoQ10 analysis each contain 30 mg/unite. Contents of three unites from each formulation were weighed separately, mixed and weight equivalent to 10 mg of CoQ10 were dissolved in 100mL CH_2Cl_2 and filtered. The resulted solution was used for analyses.

Assembled tablet formulation

Thirty mg of CoQ10 were mixed with 150 mg of magnesium oxide and 210mg of both dicalcium phosphate and microcrystalline cellulose and kept away from light at room temperature for 15 days. Equivalent weight of 10 mg of CoQ10 was dissolved in 100 mL CH_2Cl_2, filtered and used for analysis.

Chromatographic conditions

In Method I HPTLC densitometric analysis was performed on 10×20 cm glass-backed plates coated with 0.2 mm layers of silica gel 60 F_{254} (E-Merck, Germany). In Method II HPTLC densitometric analysis was performed on 10×20cm glass-backed plates coated with 0.2 mm layers of RP-18 silica gel 60 F_{254} (E-Merck, Germany) were used.

Samples were applied to the TLC plates as 6 mm bands using a Camag Automatic TLC Sampler 4 (ATS4) sample applicator (Switzerland) fitted with a Camag microlitre syringe. Application rate of 150nl/s was used. The plates were developed to a distance of 80 mm with hexane: ethyl acetate (8.5:1.5 v/v) and methanol: acetone (4:6 v/v) for Method I and Method II respectively as mobile phase in a Camag Automatic Developing Chamber 2 (ADC2) previously saturated with mobile phase vapour for 30 min at 22°C. After development and drying, the plates were scanned at 282 nm using a Camag TLC scanner IV in absorbance mode, using the deuterium lamp. The slit dimensions were 4.00×0.45 mm and the scanning speed was 20mm/s.

Method validation

The proposed HPTLC densitometric methods were validated according to the guidelines of international conference on harmonization (ICH Guideline 1996). The linearity of CoQ10 was checked between 50-800 ng/spot and 50-800 ng/spot for Method I and Method II respectively. Graph between concentration and peak area for linearity was generated. Least square linear regression analysis was applied for statistical evaluation of linearity data.

Accuracy was determined by standard addition method. The preanalyzed sample of CoQ10 (300ng/spot) was spiked with the extra 0, 50, 100 and 150 % of the standard CoQ10 and the solutions were reanalyzed in six replicates by the proposed method for Method I and Method II. The % recovery and percent relative standard deviation (% RSD) were calculated at each concentration level.

Precision of the proposed method was determined at two levels i.e. repeatability and intermediate precision. Repeatability was determined as intraday precision where three concentration (300, 400 and 500ng/spot) of CoQ10 analyzed in six replicates. Intermediate precision was determined by carrying out inter-day variation for the determination of CoQ10 at three different concentration levels of 300, 400 and 500ng/spot in six replicates for Method I and Method II on three different days.

Robustness of the proposed HPTLC method was determined to evaluate the influence of small deliberate

changes in the chromatographic conditions during determination of CoQ10 for method I and method II. Robustness was determined by changing the polarity of the mobile phase.

Limit of detection (LOD) and limit of quantification (LOQ) were determined by standard deviation (SD) method. They were determined from the slope of the calibration (S) curve and SD of the blank sample using following equations:

$LOD = 3.3 \times SD / S$

$LOQ = 10 \times SD / S$

Specificity of the proposed TLC densitometric was confirmed by analyzing and comparing the R_f values and spectra of the spot for CoQ10 in the samples with that of the standards for method I and method II.

Stability study of coenzyme Q10

Acid and base-Induced degradation

Two aliquots of CoQ10 standard (50 mg each) were dissolved in 40 mL acetone in 50 mL volumetric flasks and solutions were completed to volume with 10 mL of 1M HCl or 1M NaOH. The solutions were kept at room temperature in dark to exclude the possible degradation by light. The experiments were planned to run for 7 days. Volumes equivalent to 1 mg of CoQ10 were taken every 24 hr, completed to 10 ml with acetone in volumetric flasks and used for HPTLC analyses

Hydrogen peroxide-induced degradation

CoQ10 standard (50 mg) was dissolved in 40 mL acetone in 50mL volumetric flask and solutions were completed to volume with 10 mL of hydrogen peroxide (30.0%, v/v). The solution was kept at room temperature in dark place to exclude the possible light degradation. The experiments were planned to run for 7 days. Volumes equivalent to 1 mg of CoQ10 were taken every 24 hr, completed to 10 ml with acetone in volumetric flasks and used for HPTLC analyses.

Effect of temperature

Two aliquots of CoQ10 standard (50mg each) were dissolved in 50mL acetone in 50mL volumetric flask and one was kept in oven at 45°C for 7 days, while the other was kept at 55°C for 7 days. Volumes equivalent to 1mg of CoQ10 were taken every 24 hr, completed to 10ml with acetone in volumetric flasks and used for HPTLC analyses.

Effect of solvents

Six samples of CoQ10 standard 1 mg each were dissolved in solvent in 10 ml volumetric flasks and kept at room temperature for 7 days. The samples were analyzed for CoQ10 contents every 24 hours till the end of the experiment. Solvents used were methanol, ethyl acetate, acetone, chloroform, dichloromethane and hexane.

Quantification of coenzyme Q10 in Pharmaceutical formulations and assembled tablet formulation

The test samples were applied and chromatograms were obtained under the same conditions as for analysis of standard CoQ10. The area of the peak corresponding to the R_f value of CoQ10 standards were recorded and the amount present were calculated from the regression equation obtained from the calibration plot for Method I and Method II.

RESULTS

Method development

The maximum absorbance of CoQ10 was determined at approximately 282 nm for both Method I and Method II (fig. 1). The mobile phase was optimized to develop a suitable and accurate TLC densitometric method for analysis. The application of mobile phases composed of hexane: ethyl acetate (8.5:1.5 v/v) and methanol: acetone (4:6 v/v) resulted in sharp, symmetrical and well resolved peaks at R_f value of 0.41 (fig. 2) and R_f value of 0.28 (fig. 3) for Method I and Method II respectively. The optimized saturation time was found to be 30 min.

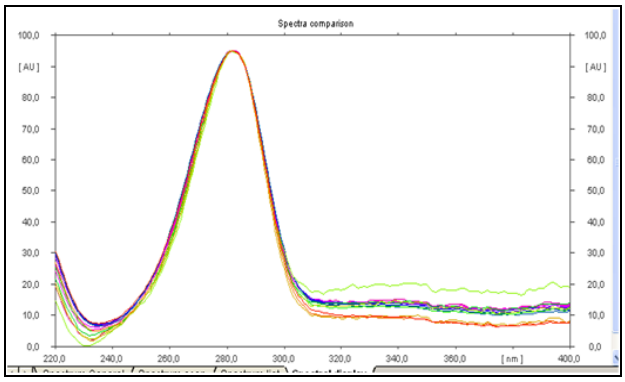

Fig. 1: Overlay UV absorption spectra of the standard and pharmaceutical formulations of CoQ10

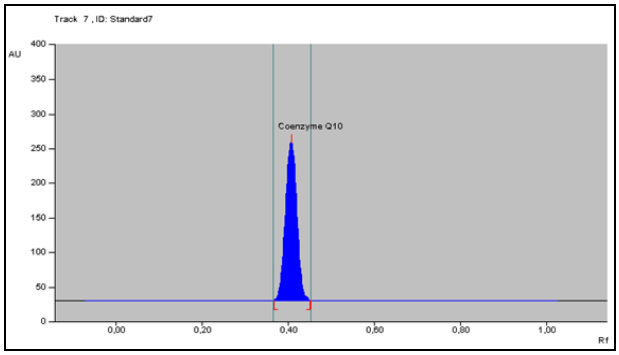

Fig. 2: HPTLC chromatogram of standard CoQ10 in Method I.

Calibration curve

The calibration plot between peak areas against concentration of CoQ10 was linear in the range of 50-800 ng/spot in Method I and Method II respectively. Linear

regression data for the plot confirmed the good linear relationship (table 1). The correlation coefficient (R^2) were 0.9989 and 0.9987 for CoQ10 in Method I and Method II respectively and was highly significant (P<0.0001). The linear regression equation was Y= 7.3826x+347.19 and Y=6.178x+236.1for CoQ10 in Method I and Method II respectively, where Y is response and X is amount of reference standards.

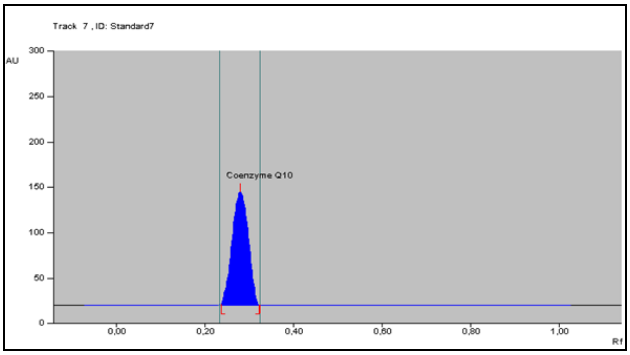

Fig. 3: HPTLC chromatogram of standard CoQ10 in Method II.

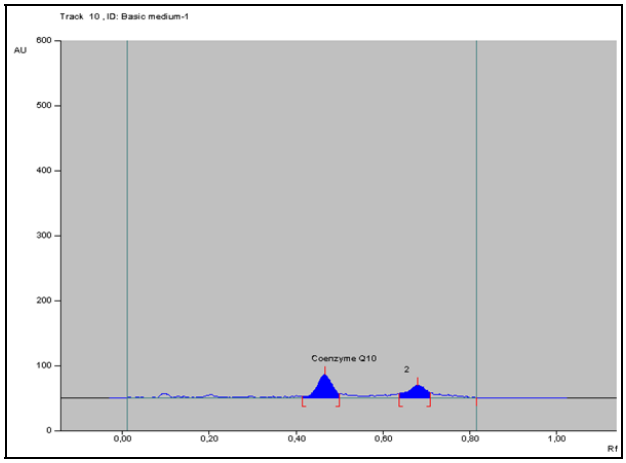

Fig. 4: HPTLC chromatogram of standard CoQ10 after 24 hours of treatment with base.

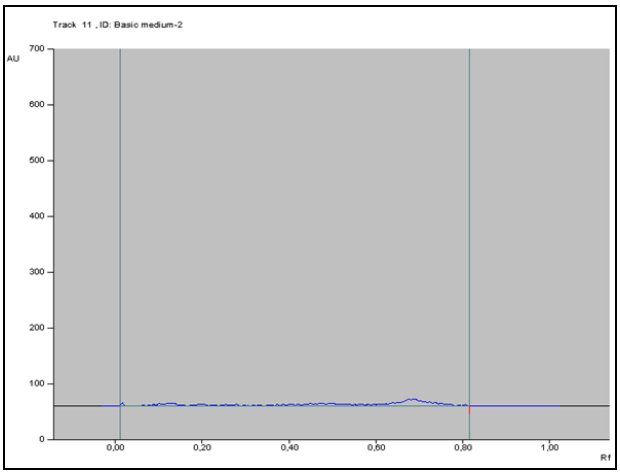

Fig. 5: HPTLC chromatogram of standard CoQ10 after 48 hours of treatment with base.

Stability study of coenzyme Q10

The chromatogram of the acid treated sample showed no effect on CoQ10. The chromatograms of the base treated sample are shown in figs. 4 and 5. The chromatograms of the hydrogen peroxide treated sample shown figs. 6 and 7.

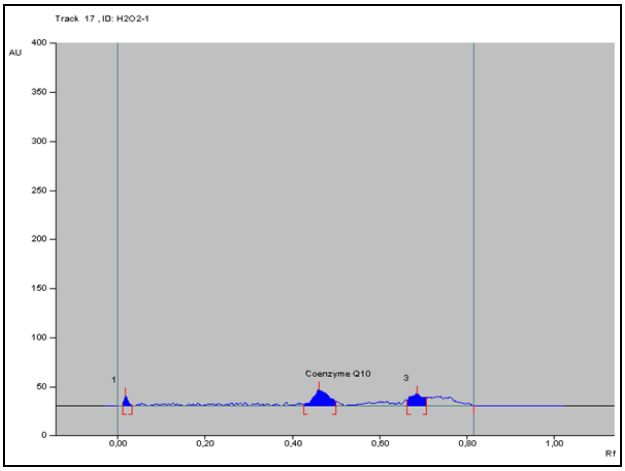

Fig. 6: HPTLC chromatogram of standard CoQ10 after 24 hours of treatment with hydrogen peroxide.

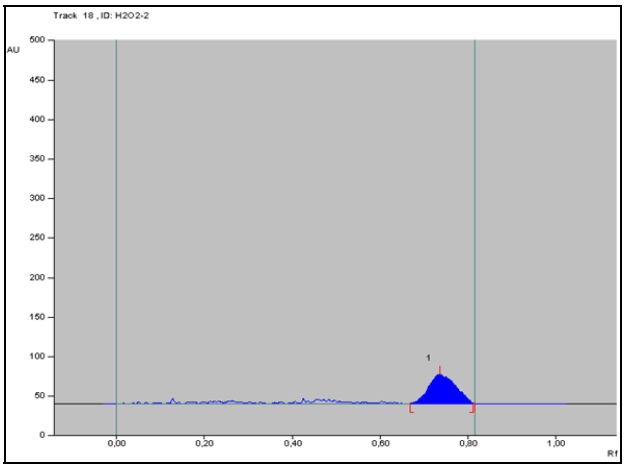

Fig. 7: HPTLC chromatogram of standard CoQ10 after 48 hours of treatment with hydrogen peroxide.

Method validation

The methods were validated via linearity (table 1), accuracy (table 2) and robustness (table 4). Both LOD and LOQ were also determined.

Quantification of coenzyme Q10 in Pharmaceutical formulations and assembled tablet formulation

Three marketed formulations were used for the analyses of CoQ10. Two products are dispensed as capsules containing 30 mg of CoQ10 and other excipients as well. The third sample was a tablet containing in addition to CoQ10, magnesium oxide, dicalcium phosphate and microcrystalline cellulose. The proposed methods were used for quantification of CoQ10 in the three products.

Table 1: Linear regression data for the calibration curve of Method I and Method II (n=6)

Parameters	Method I	Method II
Linearity range (ng/spot)	50-800	50-800
Regression equation	Y = 7.3826x + 347.9	Y = 6.178x + 236.1
Correlation coefficient	0.9989	0.9987
Slope ± SD	7.3826 ± 0.07320	6.178 ± 0.04985
Intercept ± SD	347.9 ± 34.87	236.1 ± 23.75
Standard error of slope	0.0298	0.0203
Standard error of intercept	14.23	9.69
95% confidence interval of slope	7.226 - 7.528	6.104 - 6.309
95% confidence interval of intercept	268.8 - 412.5	174.9 - 272.7
P value	< 0.0001	< 0.0001

Table 2: Accuracy of the proposed method (n=6)

Excess drug added to analyte (%)	Theoretical content (ng)	Conc. Found (ng) ± SD	% Recovery	% RSD
Method I				
0	300	295.50 ± 5.24	98.50	1.77
50	450	445.50 ± 4.85	99.00	1.09
100	600	593.33 ± 6.50	98.89	1.10
150	750	743.00 ± 7.21	99.07	0.97
Method II				
0	300	294.17 ± 4.17	98.06	1.42
50	450	444.67 ± 4.80	98.81	1.08
100	600	594.17 ± 5.12	99.03	0.86
150	750	744.67 ± 4.18	99.29	0.56

Table 3: Precision of the proposed method of Method I and Method II

	Repeatability (Intraday precision)			Intermediate precision (Interday)		
Conc. (ng/spot)	Avg Conc. ± SD (n=6)	Standard error	% RSD	Avg Conc. ± SD (n=6)	Standard error	% RSD
			Method I			
300	2627.16 ± 44.01	17.97	1.67	2615.83 ± 35.06	14.31	1.34
400	3330.66 ± 52.76	21.54	1.58	3301.33 ± 54.63	22.30	1.65
500	4225.33 ± 47.86	19.54	1.13	4196.16 ± 47.19	19.27	1.12
Method II						
300	2186.33 ± 32.31	13.19	1.48	2179.67 ± 34.05	13.90	1.56
400	2791.00 ± 28.04	28.04	1.00	2772.83 ± 31.86	13.01	1.15
500	3391.67 ± 47.46	19.38	1.40	3375.17 ± 34.82	14.22	1.03

Table 4: Robustness of the proposed HPTLC method of Method I and Method II

Method I						
Mobile phase composition (hexane: ethyl acetate)						
Conc. (ng/spot)	Original	Used		Area ± SD (n = 3)	% RSD	Rf
		8.4:1.6	-0.1, +0.1	4079 ± 68	1.66	0.42
500	8.5:1.5	8.5:1.5	0.0	4242 ± 37	0.86	0.41
		8.6:1.4	+0.1, -0.1	4229 ± 37	0.88	0.40
Method II						
Mobile phase composition (methanol: acetone)						
		3.9:6.1	-0.1, +0.1	3367 ± 39	1.14	0.27
500	4:6	4:6	0.0	3348 ± 26	0.79	0.28
		4.1:5.9	+0.1, -0.1	3345 ± 46	1.37	0.29

Table 5: Estimated and labeled amounts (%w/w) of CoQ10 in Pharmaceutical formulations by Method I and Method II.

	Method I		Method II	
	Estimated	Labeled	Estimated	Labeled
Samples	CoQ10	CoQ10	CoQ10	CoQ10
Capsule	29.45	30	29.40	30
Capsule	29.86	30	29.78	30
Tablet	23.36	30	24.96	30
Assembled Tablet Formulation	29.75	30	29.77	30

DISCUSSION

The used systems [hexane: ethyl acetate (8.5:1.5 v/v) and methanol: acetone (4:6 v/v)] for Method I and Method II respectively resulted in regular bands suitable for quantification. The proposed methods were found specific as both pharmaceutical formulation and standard showed the same R_f value. The UV spectra of the peaks were completely overlaid with a λ_{max} of CoQ10 at 282 in both Method I and Method II (fig. 1).

The relation was linear in the range of 50-800ng/spotas shown in table 1 for Method I and Method II. The regression equation were Y=7.3826x+347.19 and Y= 6.178x+236.1 with correlation coefficient (R^2) of 0.9989 and 0.9987 for Method I and Method II (table 1).

The recovery analysis afforded recovery of 98.50-99.07 % and 98.06-99.26 % for Method I and Method II after spiking the additional standard drug solution to the previously analyzed test solution indicating that the methods are accurate. Low values of % RSD (0.97-1.77) and (0.56-1.42) for Method I and Method II indicated the good accuracy of the proposed methods. The values of % recovery and % RSD are presented in table 2.

Repeatability and intermediate precision values, expressed as SD (%) are shown in table 3. RSD was in the range 1.12-1.67 and 1.13-1.65 for repeatability, 1.00–1.48 and 1.03-1.56 for intermediate precision for Method I and Method II respectively. The obtained low values are good indication for the precession of the methods.

Results of robustness study are presented in table 4. Low values of % RSD (0.88-1.66) and (0.79-1.37) for Method I and Method II were obtained after introducing small deliberate changes into the densitometric TLC procedure proved the robustness of the proposed HPTLC method.

LOD and LOQ of the proposed methods were found to be 6.29 and 19.05ng/spot and 7.50 and 22.73 ng/spot for Method I and Method II respectively. These values indicated that the proposed method can be used in wide range for both detection and quantification of CoQ10 effectively.

Literature survey revealed that all stress study regarding CoQ10 were carried out using HPLC for the effect of temperature and humidity (Estévez *et al*., 2012; Kommuru *et al*., 1999). Stability of CoQ10 was explored under different conditions. The chromatograms obtained from samples subjected to acid, base, oxidizing agent, and two different thermal stress conditions were used to assess the specificity and stability-indicating properties of the proposed methods. Acid treated sample showed no effect on CoQ10. The chromatograms showed 100% CoQ10 till the 7th day of the experiment. On the other hand, the base treated sample showed decrease in the concentration of CoQ10 to 44.6 % of the starting amount after 24 hours as determined by Method I (fig. 4). The sample analyzed after 48 hours exposure to the basic medium indicated complete disappearance of the spot corresponding to CoQ1 0 (fig. 5). Chromatogram of the hydrogen peroxide treated sample showed decrease in the spot area corresponding to CoQ10 after the first 24 hr. The percentage of CoQ10 was calculated and found to be 62.4% compared to standard solution of CoQ10 as determined by Method I (fig. 6). Analyses of CoQ10 sample after 48 hours exposure to hydrogen peroxide indicated complete disappearance of the spot corresponding to CoQ10 (fig. 7).

The samples subjected to 45 and 55°C showed no effect on CoQ10. The chromatograms showed 100% CoQ10 till the 7th day of the experiment. Samples kept in various organic solvents at room temperature for 7 days did not show any spots due to degradation or any decrease in the concentration of CoQ10.

The developed methods were applied to quantify CoQ10 in three marketed products. Two products are formulated as capsules containing other excipients. The applied method of extraction could efficiently dissolve the active ingredient from those inert excipients and no interference was noticed in the analyses. The estimated concentrations of CoQ10 were consistent with the labeled amounts in both methods with a very little variation (table 5).

The third sample was a tablet containing in addition to CoQ10, magnesium oxide, dicalcium phosphate and microcrystalline cellulose. Application of the two methods on the third formulae indicated the presence of less amount ofCoQ10 than labeled (table 5). To exclude

the possibility of interaction between ingredients the same tablet components were mixed together and kept for 15 days at room temperature away from light. Analyses of this assembled tablet formulation under same experimental condition indicated that no loss of CoQ10 was observed. It is worth to mention that the product has less CoQ10 than labeled it was no expired.

CONCLUSIONS

The proposed densitometric HPTLC methods are quite simple, accurate, highly precise, sensitive and economical. It is suitable for parallel qualitative and quantitative densitometric analysis of CoQ10 in pharmaceutical preparations. The two methods can be applied for the simultaneous routine analysis of CoQ10 samples in a relatively short time and at low cost. Moreover, it can also be utilized successfully for determination of the chemical stability and shelf life of CoQ10 in its commercial formulations, because it has stability-indicating properties. The study also revealed that CoQ10 is sensitive to alkali and hydrogen peroxide. Acid medium, temperature, magnesium oxide, dicalcium phosphate, microcrystalline cellulose and organic solvents did not show any destructive effect of CoQ10.

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REFERENCES

Aberg F, Appelkvist EL, Dallner G and Ernster L (1992). Distribution and redox state of ubiquinones in rat and human tissues. *Arch. Biochem. Biophys*., **295**: 230-234.

Bunaciu AA, Aboul-Enein HY and Fleschin S (2007). FT-IR spectrophotometric analysis of coenzyme Q10 (CoQ10) and its pharmaceutical formulations. *Prep. Biochem. Biotech.,* **37**: 59-65.

Clinical Trials. gov NCT 00096356 (2008). Coenzyme Q10 in relieving treatment-related fatigue in women with breast cancer.

Clinical Trials. gov NCT00976131 (2013). Study of CoQ10 during one cycle of doxorubicin treatment for breast cancer.

Ernster L and Dallner G (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta.,* **1271**: 195-204.

Estévez PN, Tripodi V, Buontempoy F and Lucangioli S (2012). Coenzyme Q10 stability in pediatric liquid oral dosage formulations. *Farm. Hosp.,* **36**: 492-497.

Folkers K, Hanioka T, Xia L, McReejr J and Langsjoen P (1991). Coenzyme Q10 increases T4/T8 ratios of lymphocytes in ordinary subjects and relevance to patients having the aids related complex. *Biochem. Bioph. Res. Co.,* **176**: 786-791.

Graves S, Sikorska M, Borowy-Borowski H, Ho RJH, Bui T and Woodhouse C (1998). Analysis of coenzyme Q_{10} content in human plasma and other biological samples. *Meth. Mol. Bio.,* **108**: 353-365.

Hanioka T, Tanaka M, Ojima M, Shizukuishi S and Folkers K (1994). Effect of topical application of coenzyme Q10 on adult periodontitis. *Mol. Aspects. med*., **15**: 241-248.

Hansen G, Christensen P, Tüchsen E and Lund T (2004). Sensitive and selective analysis of coenzyme Q_{10} in human serum by negative APCI LC-MS. *Analyst*, **129**: 45-50.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonised Triplicate Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption at Step 4 of the ICH Process on November 1996 by the ICH Steering Committee*,* IFPMA, Switzerland.

Kommuru TR, Ashraf M, Khan MA and Reddy IK (1999). Stability and bioequivalence studies of two marketed formulations of coenzyme Q10 in beagle dogs. *Chem. Pharm. Bull.,* **47**: 1024-1028.

Koryagin AS, Krylova EV and Luk'yanova LD (2002). Effect of ubiquinone-10 on the blood system in rats exposed to radiation. *Exp. Biol. Med.,* **133**: 562-564.

Leonard MA, Sharp RE, Darrouzet E, Moser CC, Ohnishi T, Gibney BR, Daldal F and Dutton PL (2000). Coenzyme Q oxidation-reduction reactions in mitochondrial electron transport. In Kagan, VE; Quinn, PJ. Coenzyme Q: Molecular mechanisms in health and disease. Boca Raton: CRC Press pp.65-82.

Littarru GP, Nakamura R, Ho L, Folkers K and Kuzell WC (1971). Deficiency of coenzyme Q10 in gingival tissue from patients with periodontal disease. *Proc. Natl. Acad. Sci.,* **68**: 2332-2335.

Lunetta S and Roman M (2008). Determination of coenzyme Q10 content in raw materials and dietary supplements by high-performance liquid chromatography-UV: Collaborative study. *J. AOAC. Int.,* **91**: 702-708.

McRee JT, Hanioka T, Shizukuishi S and Folkers K (1993). Therapy with coenzyme Q10 for patients with periodontal disease. *J. Dent. Heal.,* **43**: 659-666.

Michael B and Schachter MD. http://www.mbschachter. com/ coenzyme_q10.htm

Molyneux SL, Florkowski CM and George PM (2008). Coenzyme Q10: An independent predictor of mortality in chronic heart failure. *J. Am. Coll. Cardiol.,* **52**: 1435-1441.

Nakamura R, Littarru GP, Folkers K and Wilkinson EG (1974). Study of CoQ10-Enzymes in gingiva from Patients with periodontal disease and evidence for a deficiency of coenzyme Q10. *Proc. Natl. Acad. Sci.,* **71**: 1456- 1460.

Okamoto T, Matsuya T, Fukunaga Y, Kishi T and Yamagami T (1989). Human serum ubiquinol-10 levels and relationship to serum lipids. International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin- und Ernahrungs for schung. *Int. J. Vitam. Nutr. Res.,* **59**: 288-292.

Pravst I, Zmitek K and Zmitek J (2010). Coenzyme Q10 Contents in foods and fortification strategies. *Crit. Rev. Food. Sci. Nutr.,* **50**: 269-280.

Prosek M, Smidovnik A, Fir M and Strazisar M (2006). Electrochemical analysis of coenzyme Q10 and reduced coenzyme Q10. United States Patent 6984308.

Rosenfeldt FL, Haas SJ, Krum H, Hadj A, Ng K, Leong J-Y and Watts GF (2007). Coenzyme Q10 in the treatment of hypertension: A meta-analysis of the clinical trials. *J. Hum. Hypertens.,* **21**: 297-306.

Sakano K, Takahashi M, Kitano M, Sugimura T and Wakabayashi K (2006). Suppression of azoxymethane-induced colonic premalignant lesion formation by coenzyme Q10 in rats. *Asian. Pac. J. Cancer. Prev.,* **7**: 599-603.

Sándor PS, Clemente D, Coppola L, Saenger G, Fumal U, Magis A, Seidel D and Agosti L (2005). Efficacy of coenzyme Q10 in migraine prophylaxis: A randomized controlled trial. *Neurology,* **64**: 713-715.

Shindo Y, Witt E, Han D, Epstein W and Packer L (1994). Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J. Invest Dermatol.,* **102**: 122-124.

Surasi VS, Sakamoto N, Ashida N, Kishi T and Folkers K (1984). Quantitative determination of coenzyme Q_{10} in human blood for clinical studies. *Anal. Biochem.,* **142**: 155-158.

Tang PH, Miles MV, De Grauw A, Hershey A and Pesce A (2001). HPLC analysis of reduced and oxidized coenzyme Q_{10} in human plasma. *Clinical Chemist*, **47**: 256-265.

Wilkinson EG, Arnold RM and Folkers K (1976). Bioenergetics in clinical medicine. VI. adjunctive treatment of periodontal disease with coenzyme Q10. *Res. Commun. Chem. Path.,* **14**: 715-719.