ORIGINAL ARTICLE

A C G publications

J. Chem. Metrol. 10:1 (2016) 1-10

journal of chemical metrology

Comparison of qNMR and HPLC-UV techniques for measurement of Coenzyme Q_{10} in dietary supplement capsules

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(Received April 21, 2016; Revised May 30, 2016, Accepted June 25, 2016)

Abstract: The objective of this work is to apply and compare qNMR and HPLC-UV techniques as useful quality control tools for quantitative measurements of Coenzyme Q_{10} in dietary supplement capsules, for which two types of dietary supplement capsules were analysed, containing 100 and 200 mg of Coenzyme Q_{10}. Both techniques were properly validated in terms of linearity, LOD, LOQ and trueness precision (repeatability and intermediate precision) . The uncertainty of the techniques was evaluated according to per EURACHEM / CITAC Guide CG 4 (3th edition), Quantifying Uncertainty in Analytical Measurement. The HPLC-UV and qNMR methods were linear in the ranges of 10.0 –1000.0 µg mL^{-1} and 2.2 – 30.3 mg mL^{-1} for Coenzyme Q10, respectively, and demonstrated very good linearity performance with regression coefficients (R2) above ≥ 0.99. Using qNMR method, LOD and LOQ were found to be 0.48 and 1.47 mg per 0.7 ml, respectively. The LOD and LOQ values of HPLC-UV were found to be 0.025 and 0.083 µg mL^{-1}, respectively. Intra and inter batch accuracies for HPLC-UV, as a deviation between nominal and measured values, ranged from -0.3 to 7.1% and from -0.9 to 6.3%, respectively. The accuracy for qNMR was assessed using one concentration level with 6 different samples. Comparison of the measurements of the capsul samples indicated that the both methods were appropriate for the determination of Coenzyme Q10 in pharmaceutical quality control (QC), although the qNMR as a primary measurement method was found to be more convenient especially in the method development phase. The advantages of qNMR were its environmental friendliness due to the low solvent consumption, selectivity and short sample preparation time. By using the qNMR technique there is no need to concern in terms of carry over problems.

Keywords: qNMR; liquid chromatography; quantitative analysis; method development; Coenzyme Q_{10}; dietary supplements.

1. Introduction

Due to the efficacy in medical treatments, the use of nutritional supplements, such as vitamins, minerals, etc. has been an increasing health care trend in the last decade. They could be in the form of pills, capsules, powders, drinks and energy bars. Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone, is one of the most prominent nutritional supplements consumed by patients, chemical structure of which is illustrated in Figure 1.

Due to participation of CoQ_{10} in aerobic cellular respiration, generating energy in the form of Adenosine Triphosphate (ATP), this fat-soluble substance is present at high concentrations in most of the organ cells, such as heart, liver, brain and kidney cells, for the highest energy requirements [1,2]. Additionally, the use prevalence of CoQ_{10} is also due to its treatment efficiency of various diseases as

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it has strong antioxidant property. It is reported that CoQ_{10} is efficient toward some diseases such as heart [3-6], diabetes [7-10], hypertension [11-13], and neurodegenerative disease such as Parkinson's disease [14-16]. As CoQ_{10} has such widespread use related with health issues, there is a high requirement to develop robust and reliable analytical methods to determine CoQ_{10} quantitatively in dietary supplements. Hence, the present work was undertaken to develop analytical methods for quantification of CoQ_{10} in food supplement capsules, containing 100 and 200 mg of CoQ_{10}, using both liquid chromatography (LC) and quantitative nuclear magnetic resonance (qNMR) techniques. Then, the results were compared to understand the both techniques [17-19].

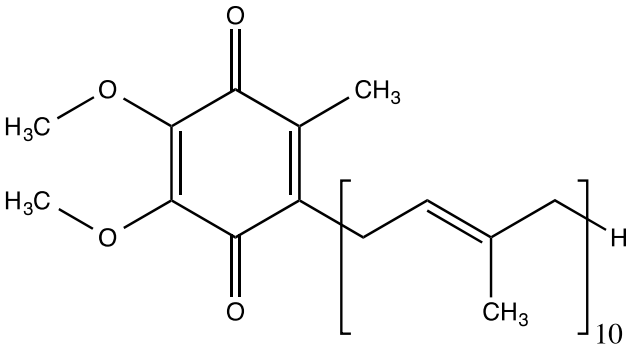

Figure 1. Chemical structure of CoQ_{10}

Nuclear magnetic resonance spectroscopy (NMR) is a very significant analytical method which has been routinely used by chemists for the determination of the structures of organic compounds. Besides, quantitative nuclear magnetic resonance spectroscopy (qNMR) has great importance due to its advantages compared to other quantification methods in many processes such as drug analysis, bio macromolecule detection, quality control of natural products and metabolic studies. These advantages can be summarized as: (i) direct proportionality of resonance signal of qNMR to the number of resonant nuclei, which makes it possible to detect analyte by using other chemical compounds as internal standard (IS); (ii) the chemical shift is related to the molecular structure, which ensures the selectivity of qNMR method; (iii) sample preparation is easy, fast, and does not need derivatization [20,21].

NMR is widely accepted as a primer measurement technique for organic compounds. In recent years, it was reported that ^{1}H-qNMR can be (applied) performed with high accuracy leading to measurement uncertainties below 1% relative. Also, the measurement of uncertainties can be reduced below 0.1% via combination of ^{1}H-qNMR with high sensitive balance weighing, using highly pure substances [22].

^{1}H-qNMR spectroscopy is a practicable method for quantitative analysis, attributed to high sensitivity of the proton nuclei, short relaxation time and nearly 100% natural abundance. Another remarkable advantage of using ^{1}H-qNMR is that there is no need for a reference standard of the same chemical structure, which is the case in chromatographic or other analytical methods [22].

2. Experimental

2.1. Chemicals and Materials

Coenzyme Q_{10} standard (98%) was obtained from Phyto Nutraceutical Inc. (Changsha City, China). Methanol was purchased from Merck KGaA (Darmstadt, Germany). 2-Propanol was obtained from Riedel-de Haën (Seelze, Germany). Supplement capsules, containing 100 and 200 mg of Coenzyme Q_{10} were obtained from MDC Pharma (Istanbul, Turkey). Filters (PTFE NS 13 mm, 0.45 Micron) were purchased from Thomas Scientific (Swedesboro, USA). Chloroform-d1 (D, 99.8%) with TMS was purchased from Merck. The Certified Reference Material (CRM) of benzoic acid with a

certified value of 99.9978 ± 0.0044% (k=2) was obtained from National Institute of Standards and Technology (NIST, USA).

2.2. Instrumentation

2.2.1. Chromatographic Conditions

An Ultra Performance Liquid Chromatography **(**HPLC) system from Agilent of 1290 series was used for the chromatographic separations. The separations were performed at a flow rate of 1.0 mL min^{-1} on Phenomenex Rex 301 column (150 mm × 4.6 mm, 5.0 µm). A binary gradient with a mobile phase consisting of methanol (A) and 2-propanol (B) was used for the HPLC separation. An isocratic gradient composed of methanol/2-propanol (40/60; v/v) was applied for 10 min. The column temperature was maintained at 25 °C using a column oven. Auto-sampler was conditioned at 4 °C. The auto-sampler syringe and the injection valve were successively washed with methanol/water (70/30; v/v) to reduce the carryover. The injected sample volume was 20 µL and detection was achieved using an ultraviolet (UV) detector set to a wavelength of 275 nm.

2.2.2. qNMR Conditions

All NMR experiments were performed at 298.15 K on a Varian VNMRS 600 spectrometer (Varian, San Francisco, CA, USA) operating at 599.747 MHz for proton (^{1}H) resonance frequency equipped with a 5 mm one NMR probe using 5 mm sample tubes (5 mm diameter, 178 mm length, Duran Group, Mainz, Germany). The software of VnmrJ 4.2 (Agilent Technologies, Santa Clara, CA, USA) was used for the data acquisition and MestReNova 10.0.0 (Mestrelab Research S.L., Santiago de Compostela, Spain) was applied for the data processing.

^{1}H-NMR experiments were conducted with 64 scans without sample spinning. The measurements were performed with the following parameters, ehich were optimized for ^{1}H-qNMR: pulse angles of 90°, 64 k data points, acquisition time of 3.4 s and auto gain function. The relaxation delay between two scans was set to 40 s. Spectral width of 9615.4 Hz and transmitter offset at 6.175 ppm were applied. Fourier transformation was done after zero filling the data to 64 k time domain points. Prior to Fourier transformation, an exponential line-broadening function of 0.3 Hz was applied to FID (Free Induction Decay) in all NMR experiments. During the data processing, phase and baseline corrections were done manually and the signals were also integrated manually for the same region. Chemical shift was referenced to the TMS signal at 0.00 ppm in chloroform-d_6. Each sample was measured 3 times, and the purities were calculated with the mean of the parallel detection results.

2.3. Sample preparation

2.3.1. Standard and QC working solution for HPLC-UV analysis

The primary standard and QC stock solutions of CoQ_{10} (1.0 mg mL^{-1}) were prepared by separate weighing in 2-propanol. All the stock solutions were sonicated for 5 min at ambient temperature. A series of standard working solutions with concentrations in the range of 10.0–1000.0 µg mL^{-1} were obtained by further dilution of the stock solution (1.0 mg mL^{-1}) with 2-propanol. The working solutions were the final concentrations of the calibrators, used to create a calibration curve, which was obtained with a series of concentrations: 10.0 (LLOQ), 20.0, 50.0, 100.0, 200.0, 500.0, and 1000.0 (ULOQ) µg mL^{-1}. A set of three different QC concentration levels, QC-low (30.0 µg mL^{-1}), QC-medium (400.0 µg mL^{-1}) and QC-high (800.0 µg mL^{-1}), were prepared by further dilution of the QC stock solution (1.0 mg mL^{-1}) with 2-propanol. All standard and QC solutions were stored in dark as short term at 4 °C for one week and as long term at -20 °C for one month. Prior to injections into the HPLC-UV system all the samples were filtered through the filters with pore size of 0.45 µm.

2.3.2. Sample preparation for HPLC-UV analysis

Sample preparation was conducted in the following manner. Each supplement capsule was opened carefully to weigh 10 mg of active substance into brown flasks, which was dissolved in 10 mL of 2-propanol. The solutions were sonicated for 5 min at ambient temperature and before injections were filtered through filters with pore size of 0.45 µm.

2.3.2. Sample preparation for method validation and ^{1}H-qNMR analysis

The weighing processes were performed on a micro balance (XP56, Mettler-Toledo GmbH, Switzerland) with a readability of 0.001 mg, certified by UME (TÜBİTAK National Metrology Institute) and checked with the class E2 weights. The balance was positioned on a 500 kg stone table, with a U-electrode to remove potential static charge. Screw cap clear glass vials with the capacity of 4 mL were used for sample preparation. Initially, an empty vial was weighed 3 times and then benzoic acid was added as an internal reference into the tube. The weighing was repeated 3 times. Finally, CoQ_{10} standard and CoQ_{10} dietary supplement were weighed in the same vial with 3 repeats. Weighing values for internal standard and CoQ_{10} were calculated with the mean of results. 1.4 mL chloroform-d6 was added into the vial and screw cap was tightly closed. The solutions were vortexed until a clear solution was obtained, after which 0.7 mL of solution was transferred into a NMR tube.

3. Results and discussion

3.1. Method Validation

3.1.1. HPLC-UV Method Validation

The parameters, which were used to validate the analytical method, were limit of detection (LOD), limit of quantitation (LOQ), linearity, carryover, trueness, precision (repeatability and intermediate precision).

3.1.1.1. LOD and LOQ of HPLC-UV Method

LOD and LOQ were estimated from the signal-to-noise ratios. The LOD was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The LOQ was defined as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten. LOD and LOQ for CoQ_{10} in diluted matrix were 0.025 and 0.083 µg mL^{-1}, respectively.

3.1.1.2. Linearity of HPLC-UV Method

The calibration curve of CoQ_{10} was established in seven levels. Three replicates of each seven standard concentrations levels were analyzed via HPLC-UV system. Calibration curves were generated using the peak areas of CoQ_{10} and were linear over the entire range measured from 10 to 1000 µg mL^{-1}. As can be noticed, the regression coefficients (R^2) were above ≥0.99 (Table 1). The calibration curve parameters obtained on each of the 3 days were suitable for the quantification of CoQ_{10} and supported the data obtained during the intra- and inter-day validation tests.

3.1.1.3 Trueness of HPLC-UV Method

The trueness was expressed as bias %. The trueness data for QCs ranged from between -0.9 to 6.3% (bias %) is represented in Table 2.

3.1.1.4 Precision (repeatability and intermediate precision) of HPLC-UV Method

Five replicates of each QC point were analyzed in a day to determine the repeatability and four replicates for the rest of the 2 days to determine the intermediate precision (in total, nine replicates). The data obtained from precision ranged from 0.5 to 1.4% (CV %). Those values met the acceptance criteria indicating that the present method was accurate and precise (Table 2).

Table 1. Calibration curve and linearity of HPLC-UV method

Compound	Calibration Range (µg mL^{-1})	Calibration equation		
		Slope mean ± SD	Intercept mean ± SD	R^2 mean ± SD
CoQ_{10}	10.0 – 1000.0	17717.53 ± 83.45	-54.32 ± 8.83	0.9999 ± 0.0001

Table 2. The accuracy and repeatability data of HPLC-UV method

Run ID	Curve No	Nominal concentration (µg mL^{-1}) of CoQ_{10}					
		30.0	%Bias	400.0	%Bias	800.0	%Bias
		Measured concentration (mg mL^{-1}) and bias (%) of CoQ_{10}					
Run-1	1	32.7	8.9	394.5	-1.4	813.1	1.6
		32.4	8.0	396.8	-0.8	813.0	1,6
		32.1	6.9	399.0	-0.2	813.1	1.6
		31.8	5.9	401.3	0.3	813.1	1.6
		31.7	5.7	402.2	0.5	811.9	1.5
Mean Concentration		32.1		398.8		812.9	
Repeatability (bias %)		7.1		-0.3		1.6	
RSD %		0.403		3.195		0.508	
Intra-Run Precision (CV %)		1.3		0.8		0.1	
n		5		5		5	
Run-2	2	31.9	6.4	392.6	-1,9	810.4	1.3
		31.7	5.7	393.0	-1.7	808.4	1.0
Run-3	3	31.5	4.9	393.6	-1.6	806.3	0.8
		31.3	4.2	394.1	-1.5	800.6	0.1
Mean Concentration		31.9		396.3		810.0	
Intermediate precision (bias %)		6.3		-0.9		1.2	
RSD %		0.439		3.669		4.289	
Inter-Run Precision (CV %)		1.4		0.9		0.5	
n		9		9		9	

3.1.1.5. Carryover

Carryover is the appearance of an analyte in a run when a blank containing no analyte is injected. Especially for the HPLC related methods, carryover is an important factor. This validation parameter must be evaluated during method development and validation. From a quantitative standpoint this parameter can be a problem and compromise the results generated from a liquid chromatography method. In the current method, a mixture of methanol/water (70/30; v/v) was used to wash syringe and

injection port couple of times before and after each injection. Under these washing conditions, the signal (area under the peak) observed on the retention time of CoQ_{10} was below 20%, compared to the one found at the LLOQ after the injection of three blank (2-propanol) samples.

3.1.2. ^{1}H-qNMR Method Validation

3.1.2.1. System Suitability of ^{1}H-qNMR

One of the advantages of ^{1}H-qNMR is that the prepared sample provides a system suitability test for of line-width and S/N data in the sample spectrum.

The Signal to Noise Ratio (SNR) of analyte signal should be more than 150 and line shape of analyte peak should be as sharp as possible. S/N ratios of benzoic acid and CoQ_{10} were 21030 and 55280, respectively, and line-width of CoQ_{10} signal was only 7.04 Hz, which indicated that the system was precise and suitable for the analysis.

3.1.2.2. Specificity and selectivity

It was obvious that the signals obtained at 8.06 ppm (2 aromatic protons) and 3.92 ppm (6 methyl protons) for benzoic acid and CoQ_{10} were not disturbed by solvent and excipients (Figure 3). Moreover, the signals of benzoic acid and CoQ_{10} were well separated from each other.

3.1.2.3. Linearity and Range of 1H-qNMR

Linearity of the ^{1}H-qNMR method was checked by preparing solutions at seven different concentrations ranging from 2.2 mg mL^{-1} to 30.3 mg mL^{-1}, according to the analyte (CoQ_{10}) in the test sample. Linearity curve was plotted taken analytes versus to foundanalytes in mg. The equation for curve was y = 0.9869x – 0.0149. The correlation coefficient was found as 1 (Figure 2). Range testing was aimed to assess the saturated concentration of the analyte in solution. Saturated solution could be prepared by adding excess analyte, but broadening of the peaks, which could result from high concentration, may influence the relaxation processes. All of our study samples and methods were valid for the whole concentration range.

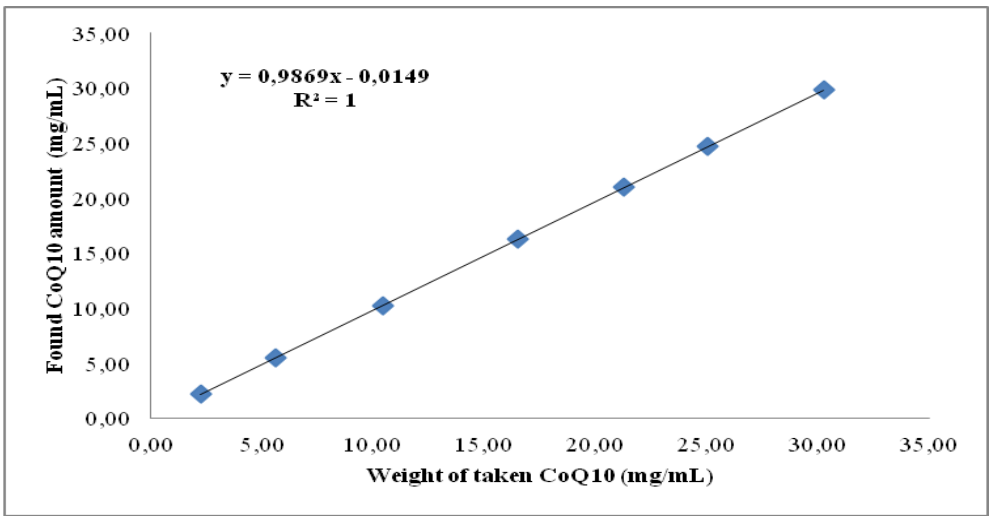

Figure 2. Representative calibration curve found and taken CoQ_{10} in mg/mL created with ^{1}H-qNMR

3.1.2.4. Accuracy of ^{1}H-qNMR

The accuracy was assessed by preparing six samples of known amount of CoQ_{10} and the IS (benzoic acid), than, analyzing the samples and comparing the gravimetric and experimental values. The average recovery of CoQ_{10} was found to be 98.968% with a standard deviation of 0.145% (Table 3).

Table 3. The test results for accuracy

	Taken (mg)	**Found (mg)**	**% Recovery**
Sample-1	33.554	33.215	98.991
Sample-2	34.691	34.414	99.201
Sample-3	34.867	34.498	98.943
Sample-4	30.879	30.514	98.818
Sample-5	31.799	31.445	98.886
Sample-6	33.763	33.482	99.167
		Mean	98.968
		SD	0.145

3.1.2.5. LOD and LOQ of ^{1}H-qNMR

LOD and LOQ were calculated by the standard deviation of the response σ and the slope S of calibration curve obtained in Linearity study, using the equations (1) and (2), respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (1) \qquad LOQ = \frac{10\sigma}{S} \quad (2)$$

LOD and LOQ were found to be 0.48 and 1.47 mg per 0.7 mL, respectively.

3.1.2.6. Precision and Intermediate Precision

Precision is defined as the degree of repeated measurements under unchanged conditions, and intermediate precision expresses variations within days. They are evaluated by RSD for repeatability. In this study, the precision was assessed by using six separate sample preparations within intraday, and intermediate precision was evaluated in three different days (Table 4).

Table 4. Precision and intermediate precision test results

	Precision			**Intermediate Precision**		
	Taken (mg)	**Found (mg)**	**Purity %**	**Taken (mg)**	**Found (mg)**	**Purity %**
Sample 1	33.554	33.205	98.96	33.554	33.215	98.99
Sample 2	34.691	34.414	99.20	34.691	34.414	99.20
Sample 3	34.867	34.501	98.95	34.867	34.498	98.94
Sample 4	30.879	30.517	98.83	30.879	30.514	98.82
Sample 5	31.799	31.455	98.92	31.799	31.445	98.88
Sample 6	33.763	33.485	99.18	33.763	33.482	99.17
		Mean	99.007		Mean	99.000
		SD	0.149		SD	0.155
		%RSD	0.151		%RSD	0.156

3.2. Uncertainty evaluation of the HPLC-UV and qNMR methods

Uncertainty is the parameter associated with the result of a measurement that characterizes the dispersion of the values that could be attributed to the measurement. The uncertainty of the HPLC-UV method was evaluated according to EURACHEM/CITAC Guide CG 4 (3th edition) titled as Quantifying Uncertainty in Analytical Measurement [23, 24]. The determined uncertainty sources for the current method are the defined parameters as following: (1) uncertainty of weighing of the starting sample, (2) uncertainty of stock solution, (3) uncertainty of calibration curve and (4) uncertainty of repeatability. qNMR uncertainty sources are (1) uncertainty of repeatability, (2) uncertainty of internal standard purity, (3) uncertainty of coenzyme Q10 molecular weight, (4) uncertainty of internal standard molecular weight, (5) uncertainty of weighing of the coenzyme Q10, (6) uncertainty of weighing of the internal standard and (7) uncertainty of integration repeatability. The relative measurement uncertainties of the HPLC-UV and Standard Measurement methods resulted in 0.64% (Table 5) and 0.134% (Table 6), respectively.

Table 5. Results from uncertainty evaluation of the HPLC-UV method

Uncertainty Sources	**x[a]**	**u(x)**	**u(x) / x**
Weighing of the Starting Sample (mg)	10.03	0.0230	0.0023
Stock Solution (mg/ml)	1000	0.8250	0.0008
Calibration Curve (C_0) (mg/ml)	809.99	0.8743	0.0011
Repeatability	1	0.0018	0.0018
Combined Standard Measurement Uncertainty (%) (u_c)		2.59	
Expanded Measurement Uncertainty (%) (U_{exp})		5.17	
Relative Measurement Uncertainty (%)		0.64	

Table 6: Results from uncertainty evaluation of the ^{1}H-qNMR method

	x[a]	**u(x)**	**u(x)/x**
Purity of CoQ_{10} (%)	99.000	0.063087241	0.000637245
Reference Purity (%)	99.9978	0.0044	4.4001E-05
M_W of CoQ_{10} (g/mol)	863.3435	0.027501333	3.18545E-05
M_W Reference (g/mol)	122.1213	0.003260695	2.67005E-05
m of CoQ_{10} (mg)	30	0.002524893	8.41631E-05
m Reference (mg)	30	0.002524893	8.41631E-05
ISample / IReference	0.7143	0.000122474	0.000171461
			0.000673283
The purity of CoQ_{10} (%)		99.000	
Combined Standard Measurement Uncertainty (%), u_{purity}		0.067	
Expanded Standard Measurement Uncertainty (%), U_{purity}		0.133	
Relative Measurement Uncertainty (%)		0.134	

[a] value

The combined uncertainty *u*(*Px*), containing additional quantities, was calculated via equation (3), according to the literature [25] for purity determinations.

$$u(P_x) = P_x\sqrt{\left(\frac{u(I_x/I_{std})}{I_x/I_{std}}\right)^2 + \left(\frac{u(M_x)}{M_x}\right)^2 + \left(\frac{u(M_{std})}{M_{std}}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(m_{std})}{m_{std}}\right)^2 + \left(\frac{u(P_{std})}{P_{std}}\right)^2} \quad (3)$$

Equation (4) was used for the calculation of uncertainty component of molar masses *u(M)*. Where; N_j indicates the number of atoms of the element j (e.g. C, H, etc.) and *u(j)* indicates the uncertainties of the atom masses.

$$u(M) = \sqrt{\sum_{j=1}^{n}\left(N_j u(j)\right)^2} \quad (4)$$

The uncertainties of the initial weighing u(mi), given by the uncertainty parameters of balance were calculated using equation (5).

$$u(m_i) = \sqrt{u_w^2(m) + 2u_{non-linear}^2(m)} \quad (5)$$

The equation of ^{1}H-qNMR for the analyte purity is as follows:

$$P_x = \frac{I_x}{I_{Std}}\frac{N_{Std}}{N_x}\frac{M_x}{M_{Std}}\frac{m_{Std}}{m_x}P_{Std} \quad (6)$$

The equation of ^{1}H-qNMR for the analyte mass is as follows:

$$W_x = \frac{I_x}{I_{Std}}\frac{N_{Std}}{N_X}\frac{M_x}{M_{Std}}m_{Std} \quad (7)$$

I_{Std}, N_{Std}, M_{Std}, m_{Std} and P_{Std} are the peak areas of number of proton, molecular weight, weighed mass and purity of the internal standard, respectively. The terms I_x, N_x, M_x, m_x, P_x and W_x indicate the peak area, number of proton, molecular weight, weighed mass, purity and mass of the analyte (Coenzyme Q_{10}), respectively. A representative ^{1}H-qNMR spectrum of CoQ_{10} is shown in Figure 3.

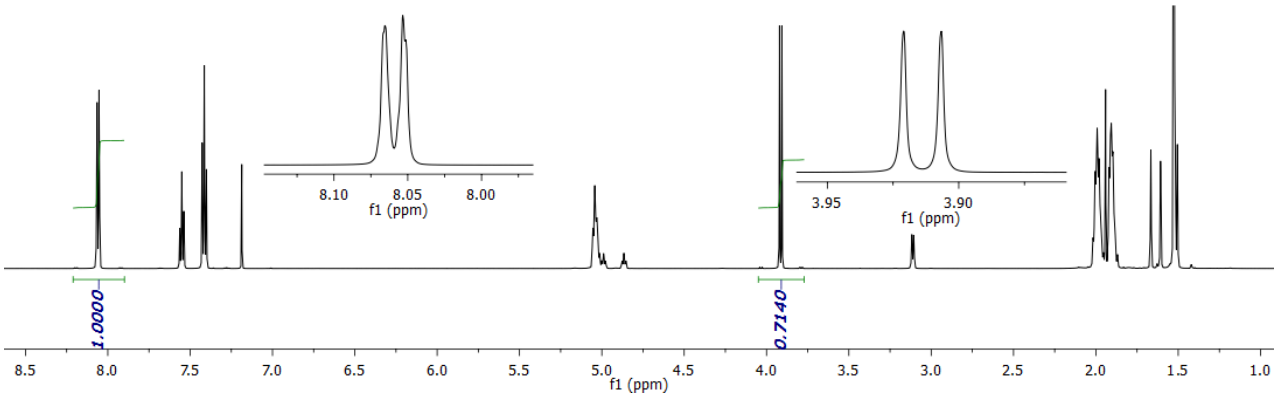

Figure 3. Representative ^{1}H-qNMR spectrum of CoQ_{10}

3.2. Application to dietary capsules using classical HPLC-UV and ^{1}H-qNMR methods

In terms of repeatability, linearity, accuracy and precision, both HPLC-UV and ^{1}H-qNMR techniques have demonstrated excellent methodological performance for the quantification of CoQ_{10} ~~in~~ dietary supplement capsules. The comparison of these two techniques reflecting very good end results of dietary supplement capsules (for capsules containing 100 and 200 mg of CoQ_{10}) is summarized in table 7.

Although both methods cover the requirements of method validation, ^{1}H-qNMR provided slightly better end results due to its better method precision. In contrast to HPLC-UV method, the sample preparation time for ^{1}H-qNMR is relative shorter, which had significant impact on the total analysis time. The advantage of ^{1}H-qNMR method indicated higher precision onto end results (see tables 5 and 6). Another advantage of ^{1}H-qNMR method is the total time needed for method optimization is relative shorter in comparison to classical HPLC-UV method. Moreover, in case of utilization of gradient, it makes the HPLC-UV method hard to transfer on another instrument. Due to all above mentioned factors, ^{1}H-qNMR can be an excellent alternative technique for the measurement of CoQ_{10} and other analytes in dietary supplement capsules. Hence, qNMR can be a more efficient quality control tool for the pharmaceutical industry in order to regulate the formulation of the pills.

Table 7: End results of both capsules containing 100 mg and 200 mg of CoQ_{10} using HPLC-UV and ^{1}H-qNMR methods

Results	**Capsules with 100 mg of CoQ_{10}**		**Capsules with 200 mg of CoQ_{10}**	
	qNMR	**HPLC**	**qNMR**	**HPLC**
Concentration (%W)	31.837	30.920	42.584	41.620
Combined uncertainty (%W)	0.232	0.488	0.096	0.369
Expanded uncertainty (%W)	0.464	0.977	0.193	0.739
Average amount of CoQ_{10} inside the capsule (mg)	**99.218**	**96.361**	**197.319**	**192.852**

4. Conclusion

The main objective of this study was to compare the quantitative performance of HPLC-UV and ^{1}H-qNMR techniques by measuring CoQ_{10} in dietary supplement capsules. Method validation for both techniques including sensitivity, bias, repeatability, inter-mediate precision and accuracy profiles was properly done, and subsequent measurement of CoQ_{10} in dietary supplement capsules using these methods was performed. Classical HPLC-UV as well as ^{1}H-qNMR techniques achieved very good results in all requested parameters, which allows for their widespread use in QC in pharmaceutical industry. In conclusion, the ^{1}H-qNMR method was designated as a slightly more appropriate technique for the determination of CoQ_{10} and its impurities in capsules due to the advantages of speed and more straightforward method development.

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