



## Electrochemical Behavior of Cholecalciferol on a Multiwalled Carbon Nanotube Modified Glassy Carbon Electrode

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**Abstract.** Herein, electrochemical behavior of cholecalciferol on a multiwalled carbon nanotube modified glassy carbon electrode was evaluated by using cyclic voltammetry. Voltammetric determination of cholecalciferol on the prepared modified electrode was carried out using linear sweep voltammetry. A good linearity was obtained with a correlation coefficient of 0.9914 between  $5 \times 10^{-5} - 1 \times 10^{-3}$  M. The LOD and LOQ values were calculated as  $1.7 \times 10^{-5}$  and  $5.1 \times 10^{-5}$  M, respectively. The response of the proposed electrode was sufficiently repeatable for determining cholecalciferol. Finally, the proposed modified electrode was successfully applied to the determination of cholecalciferol in a commercial oral solution that contains 300.000 I.U cholecalciferol/mL. A simple liquid-liquid extraction technique by using methanol was followed to extract cholecalciferol from the oral liquid. The results obtained with the proposed method were in agreement with cholecalciferol content of the commercial oral solution.

**Keywords:** Cholecalciferol, Multiwalled carbon nanotube, Voltammetry.

## Çok Duvarlı Karbon Nanotüp Modifiye Camı Karbon Elektrot Üzerinde Kolekalsiferolün Elektrokimyasal Davranışı

**Özet.** Bu çalışmada, kolekalsiferolün çok duvarlı karbon nanotüp modifiye camı karbon elektrot üzerindeki elektrokimyasal davranışı, döngüsel voltammetri yöntemi kullanılarak değerlendirilmiştir. Hazırlanan modifiye elektrot yüzeyinde kolekalsiferolün voltammetrik tayini, doğrusal taramalı voltammetri yöntemi kullanılarak gerçekleştirilmiştir.  $5 \times 10^{-5} - 1 \times 10^{-3}$  M konsantrasyon aralığında 0.9914 korelasyon katsayısı ile iyi bir doğrusallık elde edilmiştir. LOD ve LOQ değerleri sırasıyla  $1.7 \times 10^{-5}$  ve  $5.1 \times 10^{-5}$  M olarak hesaplanmıştır. Önerilen elektrodun cevabı kolekalsiferolün belirlenmesi için yeterince tekrarlanabilir. Önerilen modifiye elektrot, 300.000 I.U kolekalsiferol/mL içeren ticari bir oral solüsyonda kolekalsiferol tayini için başarıyla uygulanmıştır. Oral solüsyondan kolekalsiferolü ekstrakte etmek için metanol kullanılarak gerçekleştirilen basit bir sıvı-sıvı ekstraksiyon tekniği izlenmiştir. Önerilen yöntem ile elde edilen sonuçlar, ticari oral solüsyonun kolekalsiferol içeriği ile uyum içerisindedir.

**Anahtar Kelimeler:** Kolekalsiferol, Çok duvarlı karbon nanotüp, Voltammetri.

### 1. INTRODUCTION

Vitamin D, also known as the sunshine vitamin, is produced by the body as a response to sun exposure. It can also be consumed in food or supplements. It is a fat-soluble vitamin that is involved in regulation of calcium and phosphorus

levels in the body. Focus on the nutritional status of Vitamin D is increasing due to accumulating evidence that vitamin D is essential to human health, not only for the regulation of immune functions, but also for modification of cancer risk [1].

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Vitamin D exists in two forms: ergocalciferol (vitamin D<sub>2</sub>), derived from plants and used as a supplement, and cholecalciferol (vitamin D<sub>3</sub>), produced in the skin via a photochemical reaction with 7-dehydrocholesterol [2]. It was reported in a study that cholecalciferol is more potent than ergocalciferol in humans [3]. This has led to a drastic increase in laboratory requests for cholecalciferol measurements and an increasing need for reliable and convenient analytical methods.

Several techniques have been used for analyzing cholecalciferol such as combined thin layer and gas chromatography [4], high performance liquid chromatography [5–9], liquid chromatography-mass spectrometry (LC-MS) [10–12], supercritical fluid chromatography [13], supercritical fluid chromatography-mass spectrometry (SFC-MS) [14], voltammetry [15, 16] and bioassay [17]. Although, chromatographic methods offer the best approach to accurate determination of cholecalciferol, electrochemical techniques provide simple alternatives in this topic. Herein, voltammetric techniques were used to determine the electrochemical behavior of cholecalciferol.

Carbon nanotubes (CNTs) have attracted much attention during the past decade [18] due to their unique physical, catalytic and electrical properties. These special properties of both single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) make them ideal electrode modification materials. CNT-based electrochemical sensors in general exhibit low detection limits and fast response due to the signal enhancement provided by high surface area and rapid electrode kinetics [19, 20]. A MWCNT and poly (Alizarin red S) modified GCE was previously used for the determination of cholecalciferol [15].

In this paper, electrochemical behavior of cholecalciferol on a MWCNT modified glassy carbon electrode (GCE) was evaluated by using cyclic voltammetry (CV) and quantitative determination of cholecalciferol on the prepared electrode was carried out by using linear sweep voltammetry (LSV). The proposed electrode was successfully applied to the determination of

cholecalciferol in a commercial oral liquid. The present method is important since it has a basic surface modification process of GCE and the determination of cholecalciferol in dietary supplements can be performed accurately on the proposed electrode. The developed electrochemical technique would be a simple, cheap and sensitive alternative to the existing chromatographic methods.

## 2. EXPERIMENTAL

### 2.1. Apparatus

Voltammetric measurements were carried out by using Autolab PGSTAT128N voltammetric analyzer with a three electrode system involves a working electrode (bare GCE with a diameter of 3 mm and a geometric area of 0.0707 cm<sup>2</sup>), a platinum wire counter electrode and an Ag/AgCl (sat. KCl) reference electrode. CV and LSV were used at a scan rate 50 mV s<sup>-1</sup> between 0-1500 mV.

### 2.2. Reagents

All reagents used were of analytical grade. Cholecalciferol was obtained from Acros (Göteborg, Sweden). Methanol and ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was obtained from Merck (Darmstadt, Germany). Multi-walled carbon nanotubes (MWCNTs) were purchased from Aldrich (purity > 95%, diameter 7-15 nm, length 10 μm) (St. Louis, MO, USA) and ortho-phosphoric acid (o-H<sub>3</sub>PO<sub>4</sub>) was obtained from J.T.Baker (Phillipsburg, NJ, USA). A stock solution of cholecalciferol at 10<sup>-2</sup> M was prepared in ethanol.

### 2.3. Pre-treatment of MWCNT and preparation of MWCNT/GCE

The MWCNT was prepared as described in the previous study [21]. 40 mg MWCNT was boiled in an adequate amount of HNO<sub>3</sub>. Acid-treated MWCNT was rinsed with ultrapure water. Then a suspension was obtained by dispersing 37 mg of purified MWCNT in 3.7 mL dimethylformamide. GCE was cleaned by polishing on a synthetic cloth with Al<sub>2</sub>O<sub>3</sub> slurry, followed by ultrasonication of

the electrode for 5 min in pure water. Finally, for MWCNT/GCE, 10  $\mu$ L of the suspension were deposited on the GCE surface and dried under a 150 watts infrared lamp for 20 min.

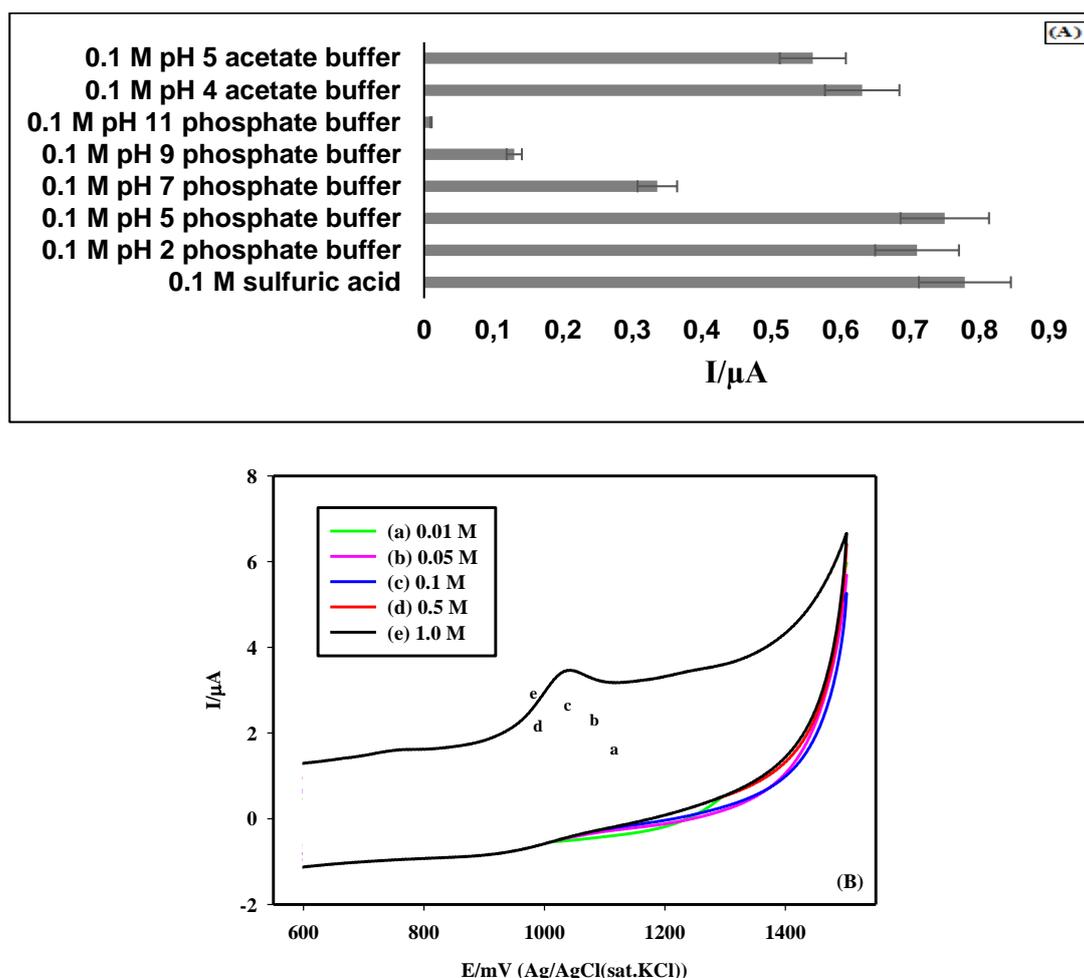
#### 2.4. Liquid-liquid extraction of cholecalciferol

A commercial oral solution that contains 300.000 I.U (7.5 mg cholecalciferol/mL) was extracted directly using 1 mL of methanol. Then, the methanol phases were filtered through a 0.45  $\mu$ m syringe filter prior to analysis.

### 3. RESULTS AND DISCUSSION

#### 3.1. Optimization of supporting electrolyte solution

Due to the insufficient solubility of cholecalciferol in aqueous medium, 1:9 (v/v) aqueous phase: methanol mixture were examined for the supporting electrolyte solution. Fig. 1A shows the effects of the aqueous phase composition on the oxidation peak currents of  $10^{-4}$  M cholecalciferol on bare GCE (n=3). It is clear that acidic media provide high peak currents. On the other hand, the CVs of  $10^{-4}$  M cholecalciferol in varying  $H_2SO_4$  solution concentrations (0.01, 0.05, 0.1, 0.5 and 1.0 M) were provided in Fig. 1B. According to the results, 0.5 M  $H_2SO_4$  solution was chosen as the supporting electrolyte solution.

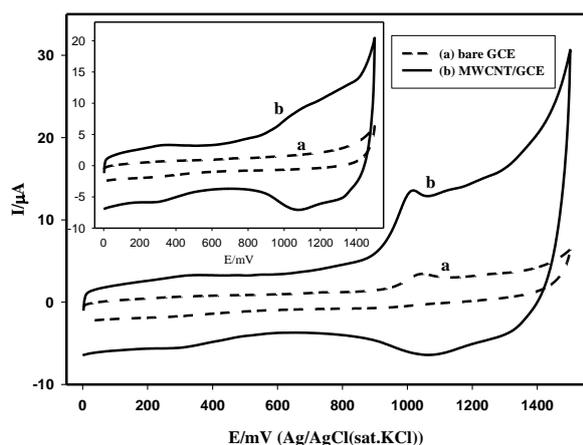


**Figure 1.** (A) The oxidation peak currents of  $10^{-4}$  M cholecalciferol in different supporting electrolyte solutions on bare GCE by using CV (n=3). Scan rate: 50  $mV s^{-1}$ . (B) The effect of  $H_2SO_4$  solution concentration on the peak currents of  $10^{-4}$  M cholecalciferol on bare GCE by using CV. Scan rate: 50  $mV s^{-1}$ .

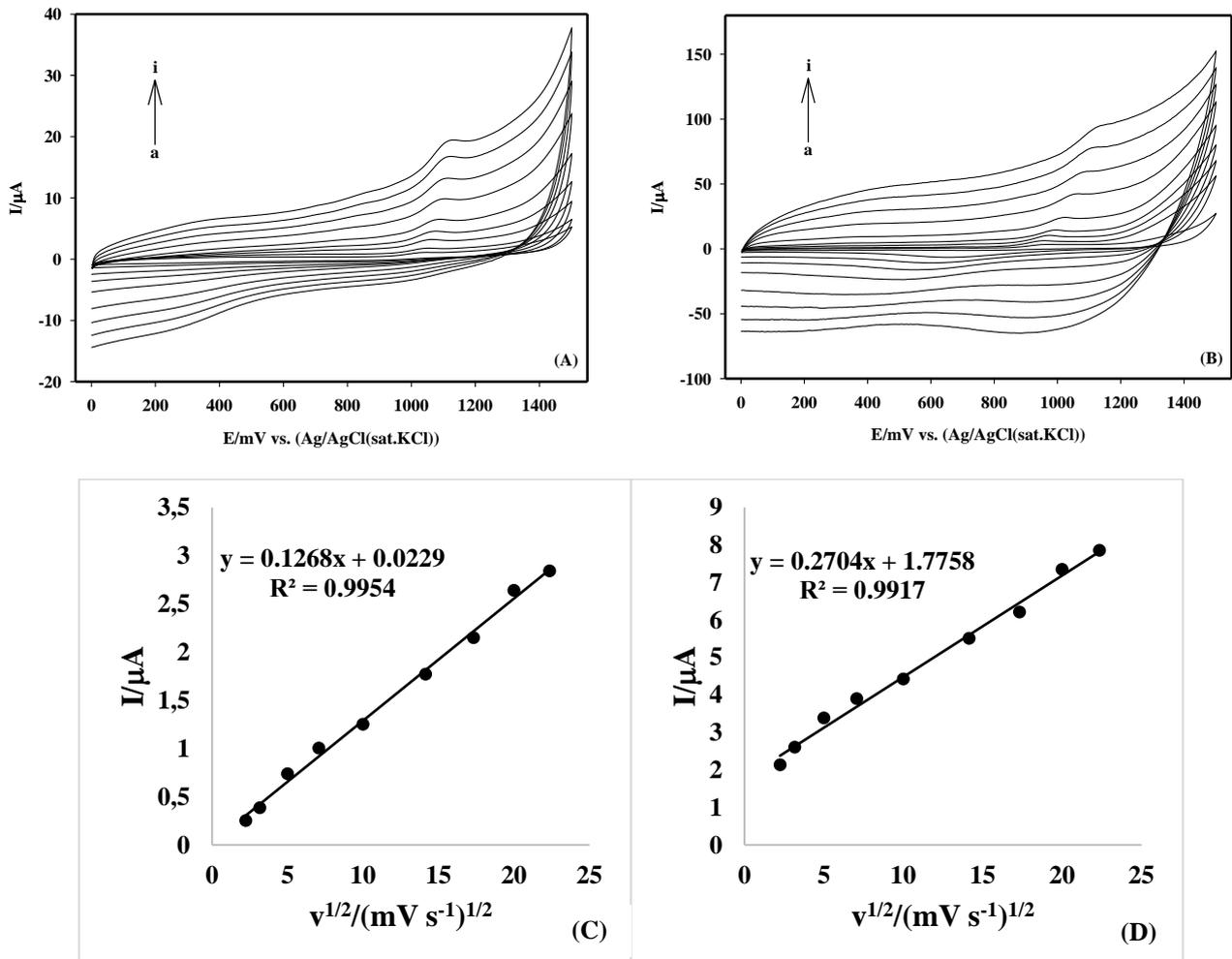
### 3.2. Cholecalciferol oxidation on MWCNT and bare GCE

The CVs of  $10^{-4}$  M cholecalciferol at optimized operation conditions on bare GCE and MWCNT/GCE were shown in Fig. 2. Irreversible oxidation peaks for cholecalciferol were observed at approximately 1040 and 1020 mV on bare GCE and MWCNT, respectively. Compared with the bare GCE, the oxidation peak current of cholecalciferol increased about six times on MWCNT/GCE. The increase of current response to cholecalciferol can be attributed to the high surface area of MWCNTs and the increase of the electron transfer process. The results indicated that modification of the electrode surface with MWCNT increases the sensitivity in the determination of cholecalciferol in the proposed method.

The CVs of  $10^{-4}$  M cholecalciferol on bare GCE and MWCNT/GCE at different scan rates was provided in Fig. 3. The effect of scan rate ( $\nu$ ) on the peak currents of cholecalciferol on bare GCE (Fig. 3A) and MWCNT/GCE (Fig. 3B) was investigated in the range of 5–500  $\text{mV s}^{-1}$ . The results showed that oxidation peak current of cholecalciferol was proportional to the  $\nu^{1/2}$  (Fig. 3C and 3D). As a result, the reactions in both electrodes are diffusion-controlled processes.



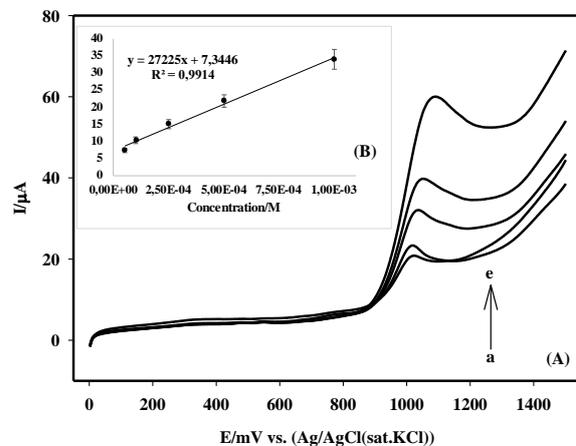
**Figure 2.** CVs of  $10^{-4}$  M cholecalciferol on (a) bare GCE, (b) MWCNT/GCE. Inset showed background CVs (a) GCE, (b) MWCNT/GCE. Scan rate:  $50 \text{ mV s}^{-1}$ .



**Figure 3.** Cyclic voltammograms of  $10^{-4}$  M cholecalciferol at various scan rates (a-i): 5, 10, 25, 50, 100, 200, 300, 400 and 500  $\text{mV s}^{-1}$  on (A) bare GCE, (B) MWCNT/GCE. The plot of  $I$  vs.  $v^{1/2}$  for the oxidation of cholecalciferol on (C) bare GCE, (D) MWCNT/GCE.

### 3.3. Linear range and sensitivity

Determination of cholecalciferol on the proposed MWCNT/GCE was carried out by using LSV. Serial standard solutions of cholecalciferol were analyzed to evaluate the linearity of the proposed voltammetric method and oxidation peaks were observed at about 1020 mV on MWCNT/GCE (Fig. 4A). As shown in Fig.4B, the proposed MWCNT/GCE was found to be linear in the range of  $5 \times 10^{-5} - 1 \times 10^{-3}$  M with a correlation coefficient of 0.9914.



**Figure 4.** (A) Linear sweep voltammograms of various concentrations of cholecalciferol on MWCNT/GCE; (a-e):  $5 \times 10^{-5}$ ,  $1 \times 10^{-4}$ ,  $2.5 \times 10^{-4}$ ,  $5 \times 10^{-4}$  and  $1 \times 10^{-3}$  M. Inset showed (B) the calibration curve on MWCNT/GCE ( $n=3$ ). Scan rate:  $50 \text{ mV s}^{-1}$ .

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the formula  $LOD=3SD/b$  and  $LOQ=10SD/b$ , respectively, where SD is the standard deviation of the response of a low concentration determinations and b is the slope of the calibration curve. The LOD and LOQ of the proposed MWCNT/GCE were calculated as  $1.7 \times 10^{-5}$  and  $5.1 \times 10^{-5}$  M, respectively.

**Table 1.** The responses of some cholecalciferol sensors.

Electrode	Method	LOD ( $\mu$ M)	LR ( $\mu$ M)	Ref.
GCE/MWCNT/poly(ARS)	SWV	5	8-160	[15]
Rotating GCE	DPV	2	2-200	[22]
GCE	ASV	3	10-40	[23]
MWCNT/GCE	LSV	17	50-1000	This work

GCE, glassy carbon electrode; MWCNT, multiwalled carbon nanotube; ARS, Alizarin red S; SWV, square wave voltammetry; DPV, differential pulse voltammetry; ASV, adsorptive stripping voltammetry; LSV, linear sweep voltammetry; LOD, limit of detection and LR, linear range.

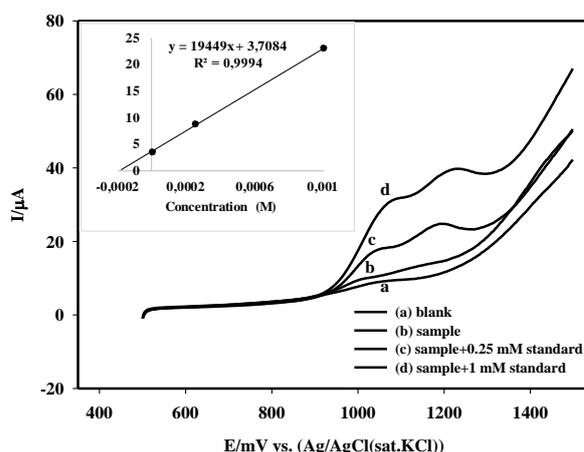
### 3.4. Precision

LSV analysis of the calibration standards at two different concentrations of  $5 \times 10^{-5}$  (low level) and  $1 \times 10^{-4}$  M (high level) were used to determine the repeatability of the proposed MWCNT/GCE. For this purpose, eight repetitions of each concentration were analyzed on the proposed MWCNT/GCE. The relative standard deviations (RSDs) of the low and high level concentrations were calculated as 8.4 and 8.5%, respectively. As a result, the response of the proposed electrode was repeatable for determining cholecalciferol.

### 3.5. Real sample analysis

Cholecalciferol oral solution 300.000 I.U (7.5 mg/mL) was extracted directly using 1 mL of methanol. Then, the sample was filtered through a 0.45  $\mu$ m syringe filter prior to voltammetric analysis. A 100  $\mu$ L aliquot was pipetted into the the voltammetric cell. Fig. 5 shows the standard addition (2.5  $\times 10^{-4}$  and 1  $\times 10^{-3}$  M cholecalciferol) LSVs obtained for the sample. The result obtained as 7.3  $\pm$  0.6 mg/mL was in agreement with cholecalciferol content of the commercial oral solution.

Comparison of electrodes previously reported for the determination of cholecalciferol is provided in Table 1. Although the sensitivity of the proposed method is relatively low, it is quite sufficient to determine cholecalciferol in dietary supplements with a broad linear range.



**Figure 5.** Linear sweep voltammograms (a) sample, after addition of (b)  $2.5 \times 10^{-4}$  M, (c)  $1 \times 10^{-3}$  M cholecalciferol standard and the standard addition calibration curve is given in inset. Scan rate: 50  $\text{mV s}^{-1}$ .

## 4. CONCLUSION

In present work, a MWCNT/GCE was fabricated for the determination of cholecalciferol by using linear sweep voltammetry. The proposed modified electrode has increased the sensitivity of the voltammetric technique for determining cholecalciferol when compared to the bare electrode. The method was linear between  $5 \times 10^{-5}$  –  $1 \times 10^{-3}$  M with a detection limit of  $1.7 \times 10^{-5}$  M.

Although, chromatographic techniques offer the best approach to accurate determination of cholecalciferol, the proposed method is more rapid and cheaper in comparison with the classical techniques. As a result, the proposed method would be a good alternative for determining cholecalciferol in dietary supplements.

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