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# Spectrophotometry, potentiometry and HPLC in determination of acidity constant for cabergoline and tadalafil

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**ABSTRACT**: Acidic dissociation constant (pKa) is an important physicochemical parameter in absorption, dissociation and elimination mechanisms of drugs in body. Various analytical methods are utilized for the determination of pKa values of pharmaceutical active ingredients, and potentiometry, spectrophotometry and HPLC are the most common methods. Cabergoline is dopaminergic ergoline derivative having a powerful and long-term prolactin reducing effect, which is used for the treatment of Parkinson disease. Tadalafil leads an increasing cGMP level in Corpus cavernous, during secretion of nitric oxide in sexual arousal. In the presented study, detection of pKa values for Cabergoline and Tadalafil by using potentiometry, spectrophotometry and HPLC was investigated. The pKa value for Cabergoline was respectively found to be 6.42, 6.05 and 6.20 by spectrophotometry, potentiometry and HPLC. Spectrophotometric pKa value was significantly different ($p>0.05$) from others, and potentiometry and spectrophotometry were appropriate for pKa value determination of Cabergoline. The pKa values for Tadalafil by potentiometry and spectrophotometry were found to be 3.52 and 3.44, respectively. But, in HPLC, no differentiation was observed in retention times of Tadalafil by increasing pH value of mobile phase. Developed methods for determination of pKa values for Cabergoline and Tadalafil demonstrated high repeatability values (RSD<%1). In this study, experimental pKa values for Cabergoline and Tadalafil from developed methods were compared with the values calculated by the common softwares and high-level divergences were observed.

**KEYWORDS**: Acidity constant; pKa; physicochemical parameter; Cabergoline; Tadalafil.

## 1. INTRODUCTION

In industrial pharmacia, perhaps the most important physicochemical characteristic property of active molecules is their acidity or basicity expressed by their acidic dissociation constant ($pK_a$) values. Because most molecules have acidic and/or basic functionalities, relationships between dissociation constants and structure may prove useful in drug design studies and in explaining the biopharmaceutical properties of substances. $pK_a$ is a physicochemical parameter that is important in industrial drug design and absorption, dissociation and elimination mechanism in human body. Ionize and non-ionize forms of any drug substance depend on Henderson-Hasselbach [1] equation is important in their passing into the cell, binding to plasma proteins and tissue penetration. Ionization degrees of drugs in different pH values are one of significant factors to determine in trans-passing to biological membranes in human body, pharmacodynamic/pharmacokinetic properties and way of drug application. Therefore, $pK_a$ determining is necessary because of directly affecting to solubility and passing of drug through biological membrane [2].

Cabergoline (CAB) (Figure 1a) is a dopaminergic ergoline derivative, which is strong and has long-time prolactin decreasing effect; inducing directly on D2-dopamine receptors on hypophisis lactotrophs thus inhibits secretion of prolactin. Additionally, CAB shows central dopaminergic affect due to the induction of D2 receptor in oral dose higher than dose using to decrease prolactin levels in serum. By these properties, CAB is frequently used for treatment of Parkinson's disease [3-4].

Tadalafil (TAD) (Figure 1b) is specific to cyclic guanosine monophosphate (cGMP), which is reversible and selective inhibitor of phosphodiesterize type 5 (PDE5). PDE5 inhibition by TAD leads an increase in the

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level of Corpus cavernosum cGMP during emission of nitric oxide in consequence of sexual arousal [5]. TAD is a selective and reversible inhibitor of phosphodisterase type 5 (PDE5) that is specific to cyclic guanosine monophosphate.

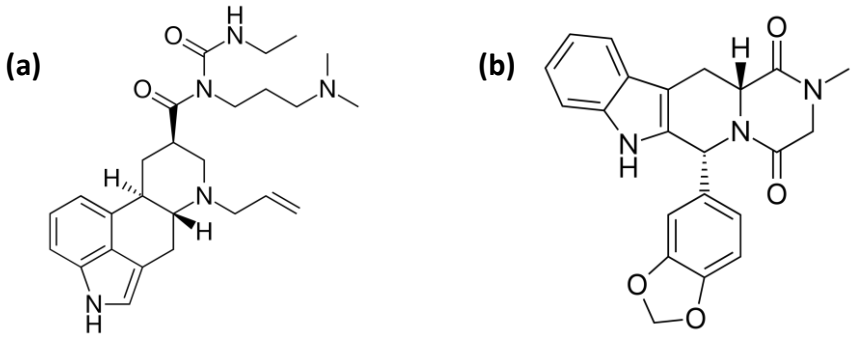


**Figure 1.** Chemical structures of (a) cabergoline and (b) tadalafil.

Different analytical methods as potentiometric, spectrophotometric and chromatographic are used for determining $pK_a$ values of pharmaceutical active ingredients. There are some limitations in determining the acidity constants of molecules such as low solubility in aqueous solutions and low values of acidity constants.

Determination of CAB from pharmaceutical preparations and/or biological matrix has been reported by using HPLC-ECD, LC-MS, LC-MS-MS and HPLC-UV [6-13], spectrophotometry [14-15] and electrochemical techniques [16]. Analysis of Tadalafil from pharmaceutical preparations using UV spectrophotometry [17-26] and 1th Degree Derivative Spectrophotometry [27, 28] are reported in the literature as spectroscopic analysis. In terms of chromatographic analysis; HPLC determinations from food and conventional pharmaceutics [29-34] , from human plasma and serum [35, 38] and from pharmaceutics [37-53] are also took place.

To the authors' knowledge, experimental $pK_a$ values for CAB and TAD are not reported in the literature. In this study, $pK_a$ of CAB and TAD by both spectroscopic and potentiometric methods were developed and optimized while cabergoline was also tested chromatographically in terms of pKa exploration.

## 2. RESULTS

### 2.1. Spectrophotometric analysis

Spectral data of CAB and TAD were taken in various pH levels of phosphate buffer (Figure 2) and the absorbance values were screened in 225 nm and 285 nm wavelengths respectively used to determine $pK_a$ values [54].

pKa values for CAB and TAD were calculated using absorbance values by Albert-Serjeant method [55] with Handerson-Hasselbalch equation (Table 1 and 2). The averaged $pK_a$ values were calculated as 6.42 ± 1.35 for CAB and 3.44 ± 0.37 for TAD.

**Table 1.** Determination of CAB calculated from absorbance values in alternating pH.

| pH | Absorbance (A) | $pK_a = pH + \log(A_I - A/A - A_M)$ |
|---|---|---|
| 3.43 | 1.3753 | 4.29 |
| 3.79 | 1.3913 | 4.37 |
| 4.77 | 1.3532 | 7.56 |
| 5.58 | 1.3854 | 6.26 |
| 6.17 | 1.3693 | 7.19 |
| 6.29 | 1.3684 | 7.34 |
| 6.49 | 1.3738 | 7.39 |
| 6.57 | 1.4044 | 6.99 |
| **$pK_a$ (Mean ± SD)** | | 6.42 ± 1.35 |

$A_m$= 1.3529 (in 0.01 M HCl), $A_i$= 1.5405 (in 0.01 M NaOH) (n=8)

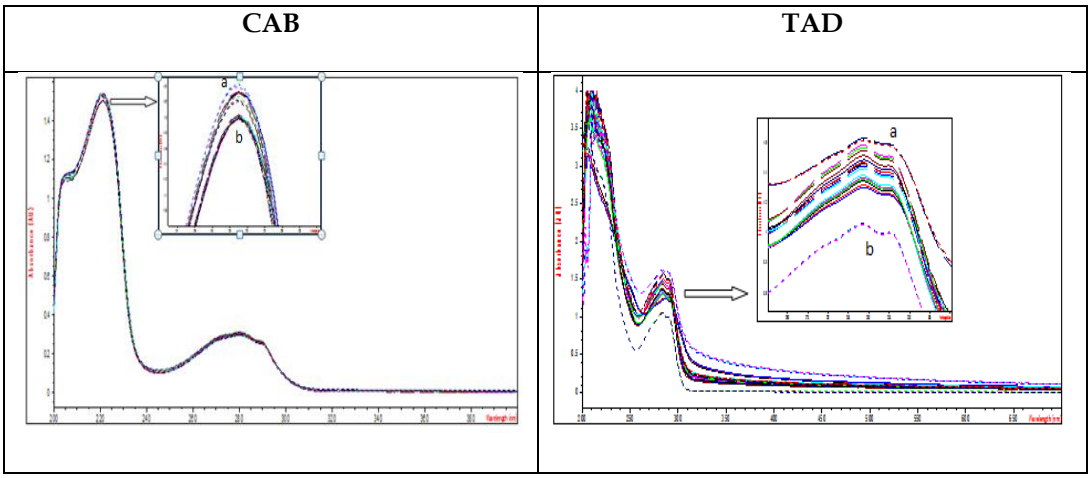


**Figure 2.** UV spectrum of CAB and TAD in different pH values of phosphate buffer and in (a) 0.01 M NaOH and (b) 0.01 M HCl solutions

**Table 2.** Determination of TAD calculated from absorbance values in alternating pH

| pH | Absorbans (A) | $pK_a = pH + \log(A - A_I/A_M - A)$ |
|---|---|---|
| 2.90 | 1.2412 | 3.17 |
| 3.14 | 1.3103 | 3.19 |
| 3.39 | 1.3539 | 3.31 |
| 3.64 | 1.4742 | 3.15 |
| 4.14 | 1.4347 | 3.80 |
| 4.93 | 1.5525 | 4.01 |
| **$pK_a$ (Mean ± SD)** | | 3.44 ± 0.37 |

$A_m$= 1.040 (in 0.01 M HCl), $A_i$= 1.6135 (in 0.01 M NaOH) (n=6)

### *2.1.1. Repeatability tests for spectrophotometric method*

Instrument and method repeatabilities test were performed for $pK_a$ analysis of tadalafil using spectrophotometric method. Tadalafil standards of 20 µg $mL^{-1}$ concentration were prepared in pH 4.5 which was close to its $pK_a$ value. The prepared solutions were measured spectrophotometically in 285 nm to test method and instrument repeatability (Table 3).

**Table 3.** Repeatability results for CAB and TAD

| | | Instrument Repeatability (n=10) | Method Repeatability (n=6) |
|---|---|---|---|
| CAB Absorbance (225 nm) | $\overline{X}$ | 1.2056 ± 0.0010 | 1.3528 ± 0.1027 |
| | SD | 0.0023 | 0.0034 |
| | RSD (%) | 0.1907 | 0.2513 |
| TAD Absorbance (285 nm) | $\overline{X}$ | 1.4687 ± 0.0037 | 1.4699 ± 0.0110 |
| | SD | 0.0090 | 0.0270 |
| | RSD (%) | 0.6128 | 1.8368 |

$\overline{X}$ : Mean ± Standart error, SD: Standard deviation, RSD: %Relative standard deviation.

## 2.2. Chromatographic analysis

In this method capacity factors were obtained against all the pH values (between pH 2.5 and 7.0). Retention time was shifting with rising of pH, and capacity factor was plotted against to pH (Figure 3). It was observed that capacity factor and asymmetry ratio were reducing by increasing organic modifier acetonitrile (ACN) content of mobile phase (Table 4).

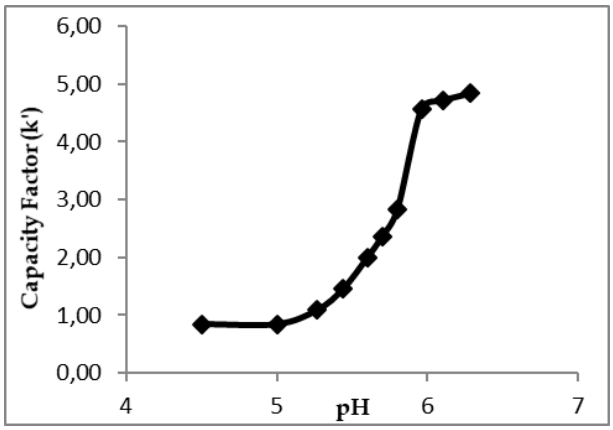


**Figure 3.** Capacity factor of CAB against buffer pH using HPLC.

Chromatographic $pK_a$ determination of CAB was monitored on the basis of retention factors increments while increasing pH, and then $pK_a$ was calculated according to the peak's capacity factor. Capacity factors calculated for CAB were plotted against to pH and the turning point of the curve would be equal to the pKa value (Figure 3).

**Table 4.** Mobile phase effect on retention time, capacity factor and peak asymmetry of CAB

| ACN/Buffer (v/v) | Retention Time (min) | Capacity Factor (k') | Peak Asymmetry Ratio |
|---|---|---|---|
| **10:90** | 6.90 | 1.30 | 5.30 |
| **15:85** | 4.77 | 0.73 | 3.11 |
| **30:70** | 3.00 | 0.28 | 1.10 |

To maintain better turning point value the second derivative of the values of the curve were plotted. pH= 6.00 where the second derivative curve passed through x-axis was found to be the $pK_a$ value of CAB (Figure 4).

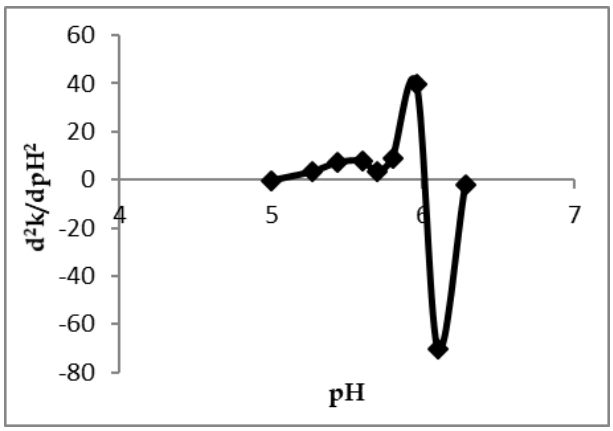


**Figure 4.** Second derivative curve of CAB.

### 2.3. Potentiometric analysis

$pK_a$ value of CAB and TAD were determined by using Gran Plot Equation with each of pH values (Figure 5).

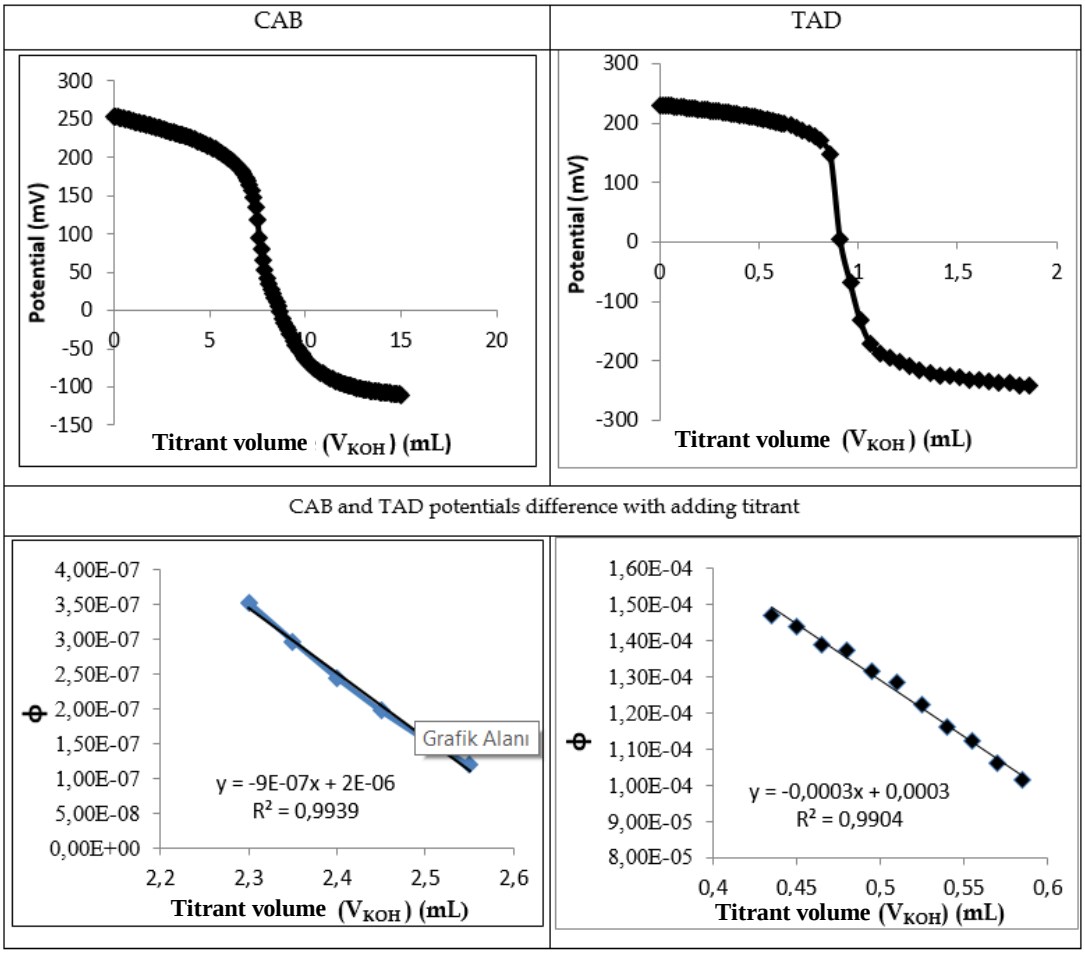


**Figure 5.** CAB and TAD Gran plot.

## 3. DISCUSSION

Among the three different $pK_a$ determination methods proposed in this study, potentiometric titration method is relatively the easiest and fastest for $pK_a$ determination. On the other hand, UV-Vis absorption spectrometry has still been used widely for the determination of dissociation constants, because of their accuracy and reproducibility. HPLC Method is effective in favorable pH range of column packing material.

**Table 5.** CAB and TAD pKa values determined by using different methods

| | CAB | TAD |
|---|---|---|
| **Potentiometric (Gran Plot)** | 6.05 | 3.52 |
| **Spectrophotometric Method** | 6.42 | 3.44 |
| **Chromatographic Method** | 6.20 | - |

In addition, potentiometric titration method was found to be much more economical and time saver for $pK_a$ determination of TAD compared to spectrophotometric method. $pK_a$ value of Cabergoline was determined by using spectrophotometric and potentiometric and chromatographic methods (Table 5). The

difference observed between $pK_a$ values with spectrophotometric and potentiometric method was statistically insignificant (Mann Whitney-U Test, $p < 0.05$).

## 4. CONCLUSION

Potentiometric and chromatographic of pKa for CAB; the spectrophotometric and potentiometric determination for TAD shows that the experimental pKa values do not match the values in the drug information cards or the software programs. A similar situation may be the case for many active drug substances whose pKa value has not been experimentally determined. For this reason, it is suggested that the pKa values of drug active substances should be determined experimentally as a physicochemical parameter that plays an important role in the mechanism of absorption, distribution and elimination of the drug in the body.

## 5. MATERIALS AND METHODS

### 5.1. Reagents

HPLC grade Acetonitrile (ACN) and Metanol (MeOH), potassium hydrogen phthalate (KHP) and uracil were purchased from Sigma-Aldrich, while analytical grade potassium dihydrogen phosphate ($KH_2PO_4$), sodium hydroxide (NaOH) and potassium hydroxide (KOH) were Ortophosphoric acid and Trietilamin (TEA) were in analytical grade (Merck, Darmstat). Ultrapure water was obtained from Barnstead NanoPure Diamond System. Cabergoline (CAB) was purchased from Pharmacia, Turkey and Tadalafil (TAD) was kindly donated by Zentiva, Turkey.

### 5.2. Preparation of the standards

Potentiometric analysis: CAB and TAD stock solutions (4000 µg $mL^{-1}$): CAB or TAD standards were weighted (20.0 mg for each) and dissolved in a 5 mL of volumetric flask with methanol separately.

Spectrophotometric analysis: In each measurement 20 µg $mL^{-1}$ CAB or TAD solutions prepared in phosphate buffer at different pH values with the same ionic strength were used. The absorption values of these solutions were measured at 280 nm wavelength at room temperature (25°C).

Chromatographic analysis for CAB: CAB solution (20.0 µg $mL^{-1}$): CAB stock solution was taken into a vial (20 µL, 1000 µg $mL^{-1}$) and end up with mobile phase to 1 mL.

Uracil solution (10.0 µg $mL^{-1}$): 10 µL of 1000 µg $mL^{-1}$ uracil stock solution was taken into a vial and end up with mobile phase to 1 mL. Uracil was used for dead volume indicator in chromatographic analysis.

### 5.3. Instrumentation

Potentiometric experiments were performed by a pH-Meter (MettlerToledo MA 235 - Glass electrode) and spectrophotometric experiments were carried out using UV-Vis Spectrophotometer (Agilent 8453). Chromatographic experiments were carried out using a Thermo HPLC system constructed with Thermo P2000 binary pump, Thermo AS3000 C autosampler, Thermo UV6000LP diode array detector, and ChromQuest Thermo Finnigan data analyzer.

### 5.4. Experimental

*5.4.1. Spectrophotometric analysis*

Spectrophotometric titration has been utilized as an alternative to determine $pK_a$ values of substances with large molar absorptivities because of its high sensitivity at concentrations of substance as low as $10^{-6}$ M. Moreover, it can handle compounds with lower solubility. However, in such a case, a compound must contain a UV-active chromophore close enough to the site of acid-base function in the molecule. The absorption spectra of the sample changes during the course of the titration by reflecting the concentration of presented neutral and ionized species.

UV-VIS sprectrum of ionized and non-ionized forms of CAB molecule prepared in 0.01 M HCl and 0.01 M NaOH solutions were taken. $pK_a$ analysis were conducted in 20 µg $mL^{-1}$ concentration level of CAB and TAD in various pH levels of phosphate buffer and the absorbance values were screened in 225 nm and 285 nm wavelengths, respectively. $pK_a$ values were calculated using Handerson-Hasselbalch equation from CAB and TAD absorbance values. Also, the repeatability of the method is checked in terms of instrument and method.

*5.4.2. Potentiometric analysis*

Potentiometric titration is one of the standard methods for $pK_a$ measurement. In a potential titration, a sample is titrated with acid or base using a pH electrode to monitor the course of titration. Potentiometric titration is a high-precision technique for determining the $pK_a$ values of substances. However, in potentiometric method, solutions of at least $10^{-4}$ M are required in order to detect a significant change in shape of the titration curve. In this study Gran's plot was used to determine $pK_a$ value. When a monoprotic weak acid is titrated by a strong base, the Gran's plot [56] is expressed by the following equation:

$$[H^+]\ V_B = K_a\ (V_E - V_B) \qquad \text{(Eq.1)}$$

where $V_E$ and $V_B$ are the volume of base added at equivalence point and at any point, respectively; $K_a$ is the acid dissociation constant. Thus, a plot of $[H^+]V_B$ vs $V_B$ will yield a linear curve having a slope equal to the $-pK_a$.

In potentiometric method, electrode was calibrated with phythalate. Potential values of Cabergoline standard solution were measured after each addition of the titrant and were plotted against to the added titrant volume (Figure 5). pH values were calculated on the basis of electrode calibration. φ values for each pH were calculated using the equation given below:

$$\varphi = (V_0 + V_{KOH}).\ 10^{+pH} \qquad \text{(Eq.2)}$$

In the experimental design of the electrode calibration using Gran plot 1.0 M KOH is prepared in methanol: water (40:60, v/v) diluent. In the first step of the calibration this titrant, including 0.1 M KCL in methanol: water (40:60, v/v) to obtain ionic strength, was used to titrate 2.94 M potassium hydrogen phosphate. In the second step of the calibration the same titrant was used to titrate 0.03 M HCl. Calibration coefficients were calculated from these experiments findings.

CAB solution (2.2 M) was titrated with 0.01 M KOH solution with the same ionic strength (0.01) provided with KCl. Titration curves were plotted using mV values against added titrant volume and pKa values were found from the calculated φ values of CAB versus titrant volume. The same procedure was applied for the potentiometric analysis in methanol: water (50:50, v/v) media.

After having calibrated the electrode; similar analysis procedure was applied for TAD with the methanol: water (60:40, v/v) as the diluent.

*5.4.3. Chromatographic analysis*

In the determination of $pK_a$ by HPLC method, the relationship between the mobile phase pH and the peak retention time of CAB was investigated. The $pK_a$ investigation of the CAB active substance was carried out with the mobile phase ACN: Phosphate buffer (10 mM, containing 0.04% TEA, 30:70, v/v) in the C18 (250 ×4.6 mm, 5 µm- Nucleodur®) column. For each pH value, firstly 20 µL of 10 µg $mL^{-1}$Uracil standard solution, then 20 µL of 20 µg $mL^{-1}$ CAB standard solution were injected into the column. Analyzes were performed at a flow rate of 1 mL $min^{-1}$ and monitored at 280 nm wavelength.

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**Author contributions:** Concept – N.E.B., A.D.; Design – N.E.B., A.D.; Supervision – N.E.B., A.D.; Materials – N.E.B., A.D.; Data Collection and/or Processing – M.B.P., N.E.B., A.D.; Analysis and/or Interpretation – M.B.P., N.E.B., A.D.; Literature Search – M.B.P., A.D.; Writing – N.E.B., A.D.; Critical Reviews – M.B.P., N.E.B., A.D.

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