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# A Developed Method for the Estimation of Diclofenac Sodium via Coupling with Diazotized 4-Aminoacetophenone

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**Abstract**

A recommended, simple and sensitive method for the spectrophotometric determination of diclofenac sodium drug (DLC) in pharmaceutical formulations was represented in this work. The method is based on the coupling of DLC with diazotized 4-Aminoacetophenone in basic medium to form an intense yellowish-brown colored diazocoupling product that showed maximum absorbance at 362 nm. The effect of various factors such as amount of the reagent, time of the reaction and order of addition were investigated. The experimental results indicated a good linearity ($r^2$ = 0.9954) over a concentration range of (2- 40) µg.mL$^{-1}$ with a detection limit of 0.447 µg.mL$^{-1}$ diclofenac sodium under the optimized conditions. The pharmaceutical excipients did not exhibit any interferences. The low value of the standard deviation alongside good correlation coefficient confirms the applicability of the method. Accordingly, the proposed method has been successfully applied to the determination of diclofenac sodium in pharmaceutical formulations. The results were statistically compared with standard methods by means of *t*-test and *f*-test at 95% confidence level with no significant differences observed.

**Keywords:** diclofenac sodium, 4-Aminoacetophenone, diazotization reaction, spectrophotometric method

## 1. Introduction

Diclofenac sodium or [2-(2,6-dichlorophenyl) amino-benzene acetic acid monosodium salt] Scheme (1), is a non-steroidal, anti-inflammatory drug (NSAID).[1,2] It is prescribed to relief pain, rheumatic and non-rheumatic inflammatory conditions. It can be administrated orally, rectally or as intramuscular injection.[2] The drug's short half-life reduces the risk of its accumulation.[3] The analgesic effect of diclofenac sodium is fast with long duration and its extensive clinical use approves a safety profile[4]. Compared to other NSAIDs the drug is well tolerated and rarely causes gastrointestinal ulceration or other serious side effects[3]. Thus, the DLC is one of the few NSAIDs that considered as the "first choice" in treating acute, chronic pain and inflammatory conditions[2]. Several analytical techniques have been used to quantify diclofenac in pharmaceutical preparations including spectrophotometry [5–12], HPLC [13-16] voltammetry [17-18] and potentiometry [19]. This work focused on the development of a simple, sensitive, selective, and interference-free spectrophotometric procedure depended on the coupling of DLC with diazotized 4-Aminoacetophenone.


**Scheme (1): The chemical structure of diclofenac sodium.**

## Experimental

### 1. Instrumentation

Shimadzu -1800 UV-Vis double beam spectrophotometer equipped with a 10 mm quartz cell for absorbance measurements, water bath (Memmert W-200 RING- Germany) and hot plate with magnetic stirrer (Germany).

### 2. Reagents

All chemicals used were of analytical reagent grade and were obtained from BDH. The reference compound of diclofenac was obtained from the State

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**Preparation of Diclofenac sodium standard stock (1000 µg.ml$^{-1}$)**

The solution was prepared by dissolving 0.1g of diclofenac sodium, in a suitable amount of methanol in 100mL volumetric flask and completing the volume up to the mark. The standard stock solution was stored at 4°C and protected from light. The working solutions were freshly prepared to be analyzed by the recommended procedure.

**Diazotized 4-Aminoacetophenone (0.005 M)**

In a 100mL beaker 67.5 mg of 4-Aminoacetophenone were dissolved in 5ml of ethanol followed by 10ml of distilled water and 1mL of 3M HCl. The mixture was heated with stirring to obtain a clear solution, then cooled to (0-5)°C in an ice-bath. 34.5 mg of $NaNO_2$ were added to the solution and stirred vigorously. Five minutes later, the solution was quantitatively transferred into a 100 mL volumetric flask and the volume was made up to the mark with cold distilled water. The solution was then stored in a refrigerator and protected from light.

**Phosphate Buffer Solution (pH 8±0.1)**

A solution of 0.1M potassium dihydrogen phosphate buffer was prepared by dissolving 13.60 g of $KH_2PO_4$ in 1000ml of distilled water, the pH value was adjusted to 8.0 by adding drops of 0.1M HCl and 0.1M NaOH solutions [20].

**Dosage form of diclofenac sodium**

**Tablets**

The content of 10 tablets of DLC (50mg/ tablet) were accurately and individually weighed and ground to fine powder, mixed well and average weight was calculated. An amount equivalent to 20.0 mg of diclofenac sodium was weighed and dissolved in a minimum volume of methanol, stirred for 10 min. to ensure complete dissolution of the drug. The solution was quantitatively transferred into 20mL standard flask and diluted with methanol to the mark to get 1000 µg. mL$^{-1}$ DLC . The sample solution was then shaken well, filtered and stored as the standard stock solution. Working solutions were freshly prepared by serial dilution.

**Ampoules**

Each injection ampoule of 3 mL contains 75.0 mg of diclofenac sodium. An accurately measured volume of 0.4 mL of the injection solution was transferred to a standard 10 mL flask and diluted to the mark with methanol to get 1000µg.mL$^{-1}$ DLC. The resulted solution was filtered and further diluted with methanol before its application.

**Results and discussion**

**Absorption spectra**

The diphenylamine group in diclofenac Sodium interacts with diazotized 4-Aminoacetophenone in basic medium to form a colored product[21]. The preliminary investigations involved the diazotization coupling of 4-Aminoacetophenone with DLC and the ability to be used in the spectrophotometric determination of the drug. A 1mL of (0.005 M) diazonium salt was coupled with 1mL of 250 µg DLC in a basic medium (1mL of phosphate buffer solution pH 8). The resulting solution was made up to a final volume of 10 mL with distilled water after 5 minutes. The absorption spectrum of the developed yellowish-brown dye was recorded against the reagent blank which showed a maximum absorbance at 362 nm figure (1).

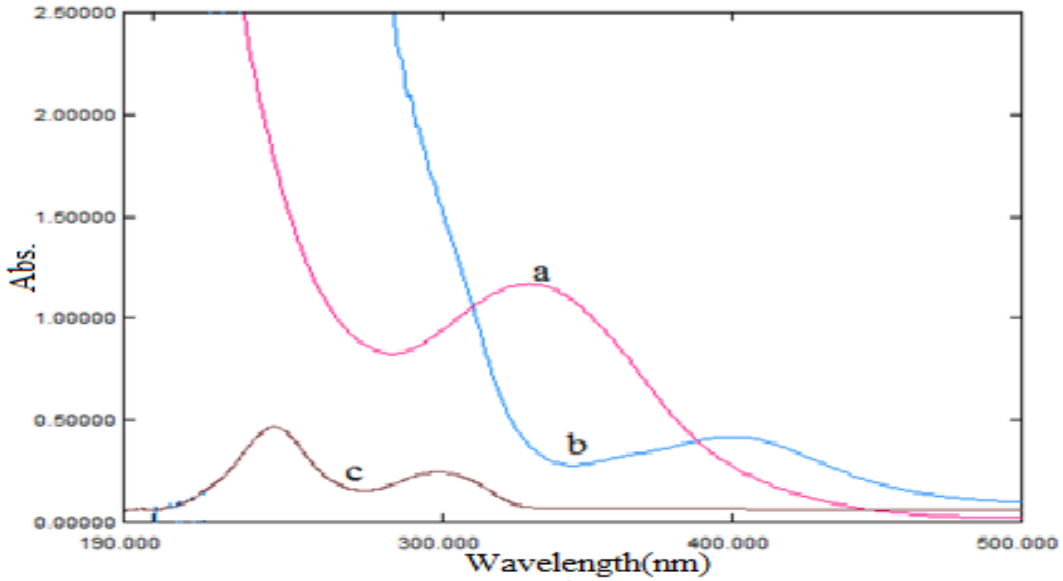


**Figure (1):** Absorption spectra**: a.** 25 µg.mL.$^{-1}$ of DLC against reagent blank under the optimum conditions, b. the reagent blank measured against distilled water, c. 25 µg.mL$^{-1}$ of DLC only against methanol.

**Influence of reagent's concentration**

The influence of diazotized 4-Aminoacetophenone concentration ranged from 0.001M to 0.05M was studied with a fixed concentration of 25 µg.mL$^{-1}$ DLC. The maximum values of absorbance was monitored and measured. It was found that the absorbance increased with increasing reagent's concentration up to 0.005M which considered as the maximum absorbance[6]. Therefore the optimum reagent's concentration chosen was 0.005M for the estimation of the cited drug figure (2)

**Influence of pH**

Effect of pH on the development of the colored product was systematically investigated within pH ranged (5.0 -12.0) ±0.02 in order to determine the optimum value of pH as shown in Figure(3). The pH was adjusted with few drops of 0.1M HCl and 0.1M NaOH. Spectroscopy measurements showed an increase in absorbance as the pH of the working solution increased. The absorbance was found to be nearly stable between pH 8.0 and 9.0. At a pH greater than 9.0, the absorption decreased immediately with the increase of pH either because of the dissociation of the complex or probably due to the formation of new species, while at pH<8.0 no reaction was observed. Therefore, pH8.0 was chosen and used in all subsequent measurements[7].

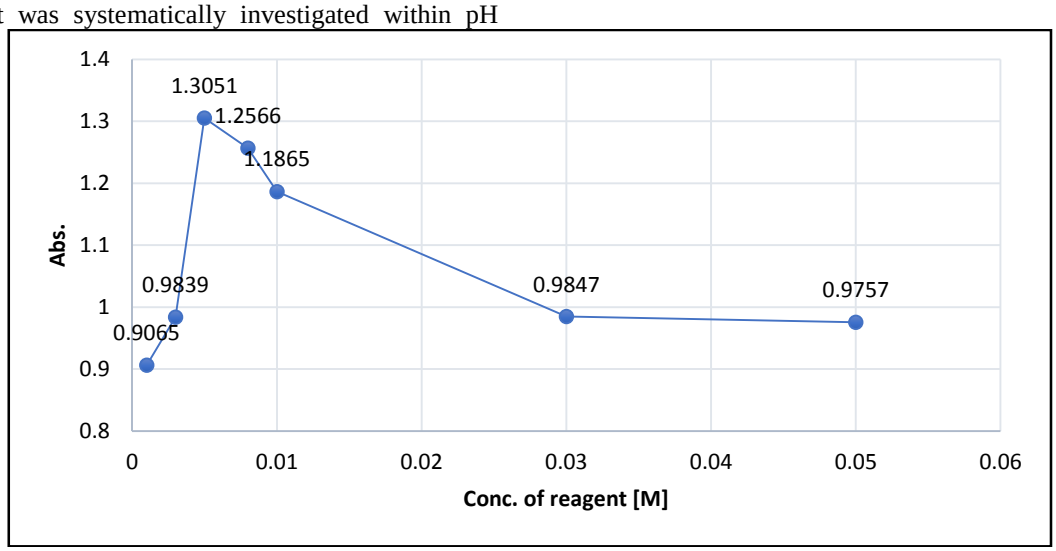


Figure (2): Effect of diazotized 4-Aminoacetophenone concentration.

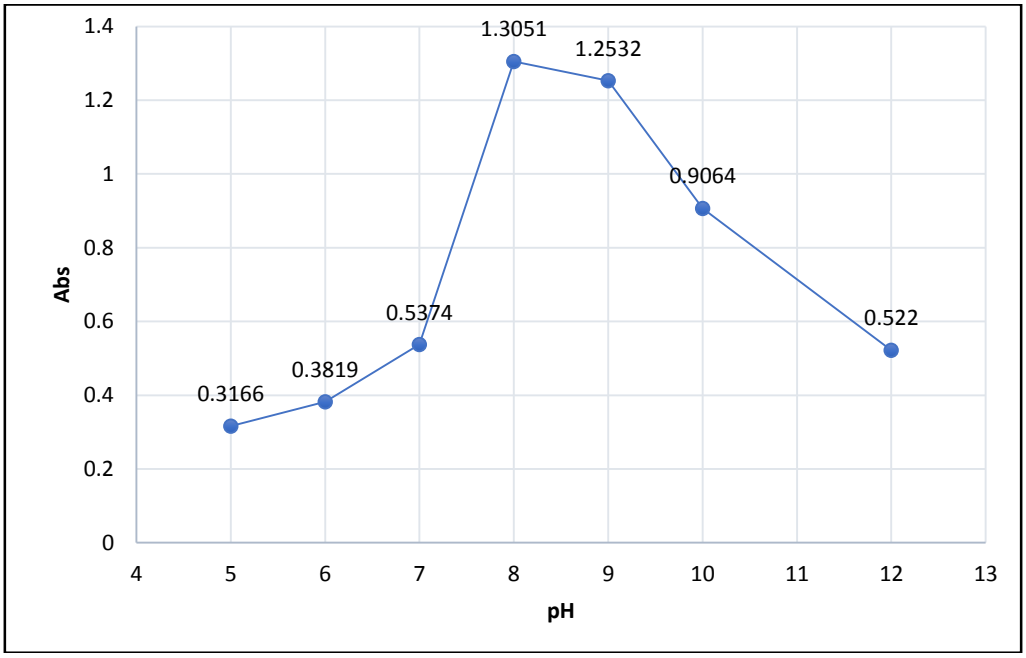


**Figure (3):** pH effect on the absorption intensity.

**Influence of coupling reaction time**

The influence of time on the coupling formation reaction of the azo-dye was tested by allowing the reaction to proceed for varying time intervals as the concentration of the product changes over time until reaching equilibrium [22]. The time required for the full coupling reaction was found to be 10 minutes, Figure (4). Leaving the reaction for longer time intervals may favor the dissociation of the azo dye and the loss in color intensity.

**Influence of order of mixing**

Different orders were used in mixing the reagents solutions. It was found that the addition of drug solution to the reagent solution followed by the addition of the buffer solution i.e. order (i) in Table (1) gave the maximum absorbance and therefore, it can be depended in the later experiments.

**Stability**

The influence of time on the color stability of the formed azo-dye was investigated by allowing the reaction product to stand for different periods of time. The intensity of absorbance reached a maximum 10 minutes after dilution to final volume and a slow decrease in absorbance value was noticed after then. The color of the azo-dye product was nearly stable for at least 60min[7]. as shown in Figure (5).

**General procedure**

In calibrated 10 mL standard flasks, 1ml of different aliquots containing (20-400) µg of DLC were added to 1mL of 0.005M of the diazotized 4-Aminoacetophenone solution with shaking followed by the addition of 1mL of Potassium dihydrogen phosphate buffer pH= 8±0.1. After 10 min each solution was diluted to the mark with distilled water. Measure the absorbance of the colored chromogen was measured at 362 nm against the reagent blank.

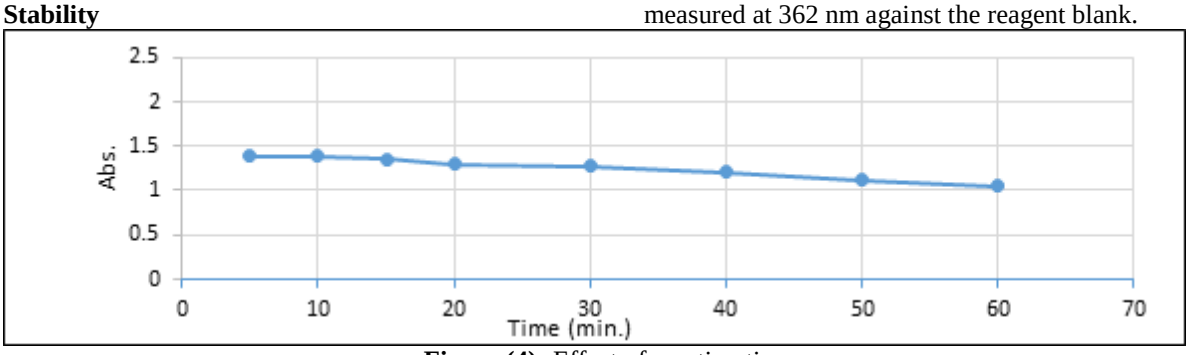


**Figure (4):** Effect of reaction time.

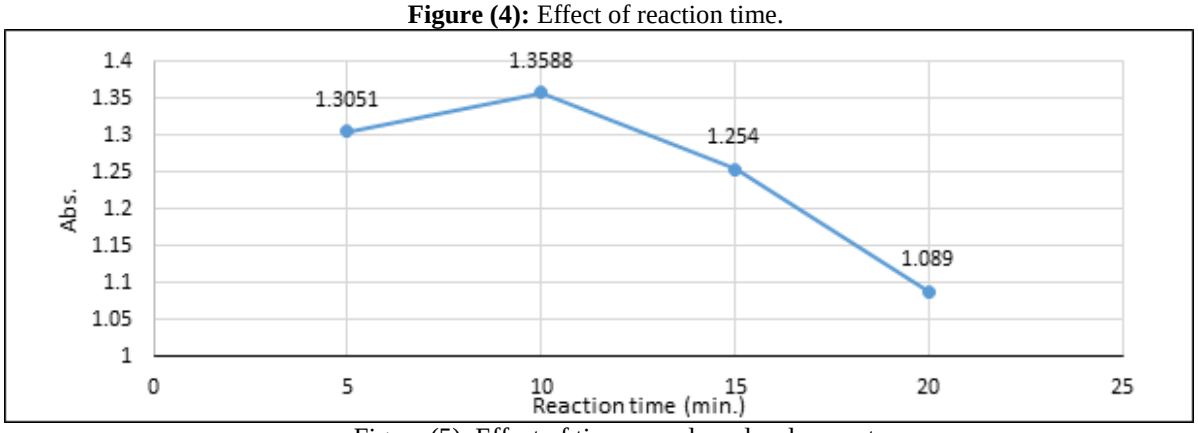


Figure (5): Effect of time on colour development

Table (1): The effect of addition order.

| Type | The order | Abs. |
|---|---|---|
| **i** | **Diazotized reagent + Drug +Buffer pH=8** | **1.3588** |
| **ii** | Diazotized reagent + Buffer pH=8+ Drug | 0.7164 |
| **iii** | Drug + Buffer pH=8 + Diazotized reagent | 0.2246 |
| **iv** | Buffer pH=8 + Diazotized reagent + Drug | 0.7300 |

**Calibration curve and analytical data**

Calibration graph was constructed using standard DCL solutions under optimum experimental conditions. A linear relationship was observed between the absorbance and concentration of DCL ranged (2.0 – 40.0) µg.mL$^{-1}$ as shown in Figure (6).

Table(2) shows the intercept, correlation coefficient, Sandell's sensitivity, molar absorptivity (ε), detection limit, and quantities limit. The high sensitivity of the proposed method was indicated by small value of Sandell's sensitivity.

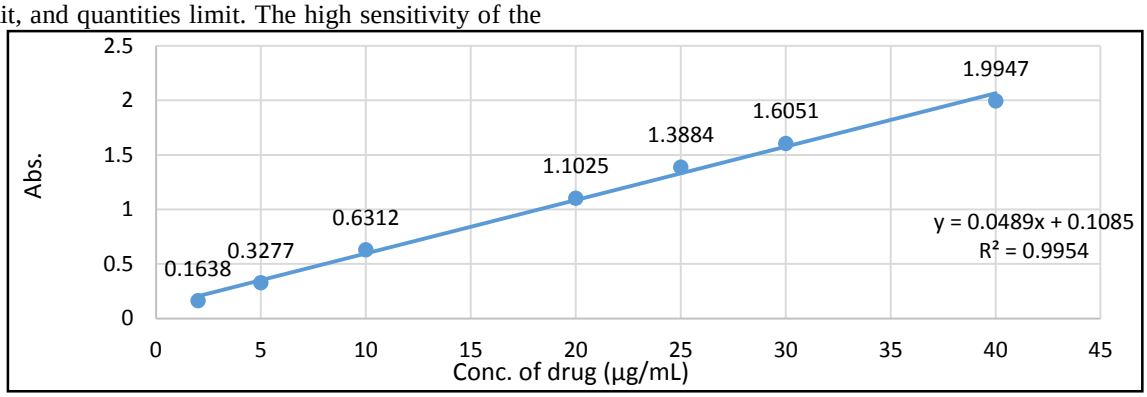


Figure (6): Calibration graph of DLC.

Table (2): Optical characteristics and statistical data for the determination of DLC.

| No. | Parameter | Value |
|---|---|---|
| 1 | $\lambda_{max}$(nm) | 362 |
| 2 | Apparent molar absorptivity (L.mol$^{-1}$.cm$^{-1}$) | $1.4481\times10^4$ |
| 3 | Sandell's sensitivity (µg.cm$^{-2}$) | 0.0204 |
| 4 | Slope (mL. µg$^{-1}$) | 0.0489 |
| 5 | Intercept | 0.1085 |
| 6 | Regression coefficient (r) | 0.9976 |
| 7 | Beer's Law Limit (Linearity, µg. mL$^{-1}$) | 2- 40 |
| 8 | Limit of detection (µg. mL$^{-1}$) | 0.4447 |
| 9 | Limit of quantitation (µg. mL$^{-1}$) | 1.4826 |

**Accuracy and precision**

The accuracy and precision of the recommended spectrophotometric method for the determination of DLC were established by calculating the relative error percent and the relative standard deviation obtained by actual determination of three replicates at three different concentration levels of the drug selected within the Beer's law limits. The results for the proposed method were recorded in Table (3).

**Applications**

Quantification of DLC in various samples of pharmaceutical preparations was carried out following the suggested procedure. The effect of excipients and additives commonly used in dosage forms of DLC was studied in the determination of 25.0 µg.ml$^{-1}$ of DLC. The results given in Table (4) shows no interference in the presence of glucose, starch, Sucrose, lactose and Mg-stearate were observed with the ratios commonly used in pharmaceutical preparations of DLC.

Table (5) shows the values of recovery percentage obtained for the analyzed samples by applying the proposed method on the pharmaceutical formulations. The results of the analysis were satisfactory.

The recommended method was statistically compared with other methods. No significant differences was found with the methods relying on t- test at 95%. On the other hand, F- test (at 95% confidence limit) shows that there is no significant differences between the recommended method and the three standard methods. All results are tabulated in Table(6)

**Table (3):** Evaluation of accuracy and precision for the determination of DLC.

| Conc. of DLC (µg.mL$^{-1}$) | | Er % | RSD % |
|---|---|---|---|
| Taken | Found* | | |
| 5.000 | 5.022 | 0.440 | 0.610 |
| 10.000 | 10.057 | 0.570 | 0.346 |
| 20.000 | 20.206 | 1.030 | 1.035 |

*Average of three measurements.

**Table (4):** Percent recovery of DLC solution in the presence of excipients.

| Excipients | Conc.of excipients. Taken(µg.mL$^{-1}$) | DLC Conc. Taken (25.0 µg.mL$^{-1}$) | | |
|---|---|---|---|---|
| | | Found (µg.mL$^{-1}$) | E rel% | Recovery% |
| Glucose | 1000 | 25.402 | 1.060 | 104.022 |
| Starch | | 25.493 | 1.097 | 104.931 |
| Sucrose | | 25.394 | 1.576 | 103.941 |
| Lactose | | 25.354 | 1.416 | 103.540 |
| Mg-stearate | 100 | 24.879 | 0.484 | 98.793 |

**Table (5):** Application of the proposed method to the DLC concentration measurements in tablet and ampoule.

| Sample | Weight* found (mg) | Concentration (µg.mL$^{-1}$) | | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| | | Taken | Found* | | |
| Diclofenac sodium 50mg/Tablet (India) | 47.520 | 5.000 | 4.752 | 95.040 | 0.538 |
| | 45.315 | 10.000 | 9.063 | 90.630 | 0.691 |
| | 49.345 | 20.000 | 19.738 | 98.690 | 0.363 |
| Diclofenac sodium 75 mg / 3 mL Ampoule (Egypt) | 66.885 | 5.000 | 4.459 | 89.180 | 0.381 |
| | 69.600 | 10.000 | 9.280 | 92.800 | 0.422 |
| | 71.700 | 20.000 | 19.624 | 98.120 | 0.572 |

*Average of three measurements.

**Table (6):** T- and F- values for analysis of 10 µg.mL$^{-1}$ DLC in pharmaceutical tablets.

| Method | Assay (mg/tablet)* | | SD | t-values | F-values | Reference |
|---|---|---|---|---|---|---|
| | *Spiked* | *Found (average)* | | | | |
| First | **10.0** | 9.063 | 0.538 | 3.065 | ------- | Proposed method* |
| Second | | 9.035 | 0.178 | 3.182 | 3.050 | 7 |
| Third | | 9.268 | 0.019 | 4.205 | 2.122 | 12 |

* DLC 10 µg.mL$^{-1}$ (India), Average of three measurements.

## Conclusion

As a result, the developed spectrophotometric method for the determination of DLC was found simple, precise, accurate, and sufficiently sensitive to be applied for the determination of small amounts of DLC in its pure and pharmaceutical dosages forms. Therefore, the proposed method could be recommended for the analysis of DLC in quality control laboratories.

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