# Chromatographic and Spectrophotometric Determination of Clindamycin in Pharmaceutical Products

**Idrees Faleh Al-Momani*** **and Lana Mohammad Zaid Al-Kilani**

*Department of Chemistry, Faculty of Science, Yarmouk University, Irbid 21163, Jordan*

** **Corresponding author:***

*email: imomani@yu.edu.jo*

*Received: December 8, 2023*
*Accepted: February 23, 2024*

**DOI:** 10.22146/ijc.91599

***Abstract:*** *Accurate, precise, and reliable chromatographic and spectrophotometric methods were developed for determining clindamycin (CLD) in pharmaceutical formulations. The spectrophotometric method was adopted for flow injection analysis (FIA). The method is based on the online oxidation of CLD and measuring the absorbance of the resulting product using a flow cell at 605 nm. Experimental conditions, including FIA variables and reaction conditions, were optimized. The chromatographic separation was achieved using a $C_8$ column and an isocratic mobile phase. The composition of the mobile phase selected for the analysis consists of a mixture of phosphate buffer (50%), MeOH (35%), and ACN (15%), adjusted to a pH of 3.47 by phosphoric acid. The eluent was monitored with a UV detector at 205 nm. The linearity range was 10–200 and 50–800 µg/mL for the FIA and HPLC, respectively. The applicability of the FIA and HPLC methods was validated by analyzing CLD in synthetic and commercial pharmaceutical products. No significant interferences were observed from the common excipients usually used in commercial formulations.*

***Keywords:*** *clindamycin; FIA; HPLC; pharmaceutical products; spectrophotometry*

## ■ INTRODUCTION

Clindamycin (CLD) is a type of antibiotic that belongs to the lincosamides group. This class of antibiotics is used widely against a variety of pathogens. The IUPAC name of CLD is methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside (Fig. 1). CLD is a semi-synthetic derivative of lincomycin and is approximately 20 times more effective than lincomycin at inhibiting bacterial growth [1]. CLD, a potent compound, is effective against many Gram-positive and Gram-negative pathogens that cause various infections. It is commonly used topically to treat acne by reducing the growth of *Propionibacterium acnes*. Moreover, it has been used to treat infections in the head and neck, respiratory system, bones, soft tissues, abdomen, pelvis, and skin [1].

There are several analytical techniques available to determine CLD in different matrices. Among these, HPLC is the recommended USP method. HPLC is accurate and specific, making it the most commonly used

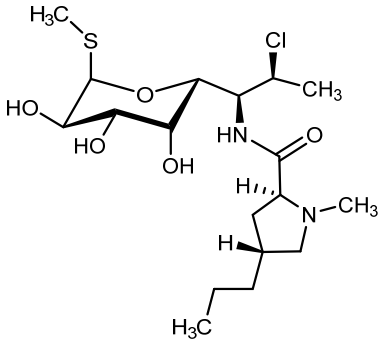


**Fig 1.** Chemical structures of CLD

method [2-9]. Other techniques including liquid chromatography-mass spectrometry [10-12], electrochemiluminescence [13], potentiometry [14], capillary electrophoresis [15], miceller chromatography [16], chemometrics [17], and spectrophotometry [18-21] are also used. But some of these techniques are time-consuming, require extraction with organic solvents, and have complex designs that require expensive equipment or specific detectors. For instance, CLD was determined spectrometrically by oxidizing the sulfur atom using potassium iodate in an acidic medium. This

process resulted in the liberation of an equivalent amount of iodine, which was then quantitatively extracted into cyclohexane after 45 min at 60 °C [18]. In a separate study, triiodide ions were detected at 350 nm within 40 min of mixing reagents [19].

Quantitative spectrophotometric analysis of CLD is limited due to the absence of any UV chromophore in its structure. The FIA method is highly efficient in conducting fast, accurate, and precise chemical analysis [22-25]. The current paper presents fast, simple, and accurate methods for determining CLD using chromatography and spectrophotometry. The HPLC method takes only 5 min for total analysis and is free of interference. The batch spectrophotometric methods are known to be time-consuming and need large quantities of reagents. Therefore, adaptation for FIA has automated the suggested spectrophotometric method.

## ■ EXPERIMENTAL SECTION

### Materials

All reagents were of high purity. The active ingredient, CLD, was provided by MAS Pharmaceuticals Industries (Amman, Jordan). The commercial pharmaceutical products were purchased from the local market. The organic solvents used for chromatographic separation were all from LAB-SCAN. Sodium-dihydrogen orthophosphate dihydrate (SHP) and phosphoric acid were purchased from MERCK.

### Instrumentation

The FIA system was set up using 0.51 mm microline tubes. It consists of two lines, the first tube pumps sodium hydroxide (NaOH) solution which carries the injected sample, as shown in Fig. 2. The second tube pumps potassium permanganate ($KMnO_4$) solution. In the reaction coil (RC), the injected CLD reacts with the pumped permanganate solution in a basic environment. To speed up the reaction, the mixture in the RC was heated by placing it in a hot water bath at a temperature of 80–90 °C. The absorption of the green product was monitored at 605 nm using a SPUV-19 UV-visible instrument. When the absorbance change has reached its maximum after injection, the valve is reset to the load position. The HPLC system was the KNAUER model-501 LC connected to a Knauer K-2501 UV detector. Samples were presented by an injector with a 20 µL loop and the eluent was monitored at 205 nm. The chromatograms were acquired and manipulated using Eurochrom-2000 software.

### Procedure

#### *Solutions for FIA method*

$KMnO_4$ solution (3.0 mM) was prepared by dissolving 0.2371 g of $KMnO_4$ in a 500 mL volumetric flask and diluting to volume by distilled water. NaOH solution (0.4 M) was prepared by dissolving 8 g of NaOH in 500 mL of distilled water. Active ingredient standard solution (CLD) of a stock solution of 1000 µg/mL of CLD was prepared by dissolving 0.10 g of the pure CLD in 5 mL of 0.10 M HCl and diluting to volume with distilled water. More dilute solutions were prepared by successive dilution. Pharmaceutical product samples were purchased from the local market, whereas three commercial products containing CLD in tablet form, namely Clinimycin, Clindamyl, and Clindacin. Twenty tablets of the commercial product Clinimycin (150 mg CLD/tablet) were mixed and an amount equivalent to one tablet was placed into a 100 mL volumetric flask. Then, 5 mL of 0.10 M HCl was added, and the mixture was placed in an ultrasonic shaker for 5 min. The solution was then diluted to 100 mL with distilled water.

#### *Solutions for HPLC method*

An internal standard (IS) solution of ciprofloxacin was used. A stock solution of 400 µg/mL was prepared in distilled water. The concentration of the IS solution was kept constant at 20 µg/mL for all samples. The same procedures were followed for the preparation of the CLD standard solution and commercial tablets as in the FIA method. However, 2.5 mL of the IS solution was added to each solution before reaching the final volume. The concentration of the IS was maintained at a constant value of 20 µg/mL in the final solution for all cases.

#### *Chromatographic conditions*

The separation was achieved using a reversed-phase $C_8$ column (RP-$C_8$) column from Varian with

dimensions of 15 cm × 4.0 mm ID and a particle size of 5 µm. The mobile phase consists of a mixture of phosphate buffer (50%), MeOH (35%), and ACN (15%), adjusted to a pH of 3.47 by phosphoric acid. The mobile phase was filtered and degassed before use.

## ■ RESULTS AND DISCUSSION

### FIA Method

It is essential to account for several factors while measuring CLD concentrations at its maximum absorption peak of 205 nm. Firstly, several interferences in the matrix would interfere with the wavelength at which CLD absorbs. Secondly, the chemical structure of CLD lacks real chromophores, leading to a weak absorption band and low detection sensitivity. Thus, it is important to consider these factors while measuring CLD concentrations.

In this study, CLD was oxidized using $KMnO_4$ in a basic medium. The color of the oxidizing agent, $KMnO_4$, changed from purple to green. The absorption maximum of the produced $K_2MnO_4$ occurred at 605 nm. The intensity of the green color produced was found to be proportional to the concentration of CLD. The reaction was affected by several factors, which were adjusted accordingly.

Several tests have been conducted to identify the most favorable conditions for the reaction between the CLD and the oxidant. Keeping the CLD constant at 100 µg/mL, different buffers, acidic and basic solutions have been examined to determine how acidity affects the reaction's completion and the analytical signal. Results indicated that the highest analytical signal was obtained in basic solutions. Therefore, different concentrations of NaOH were tested over the range from 0.05 to 1.20 M using the FIA manifold in Fig. 2. The maximum analytical signal was obtained at 0.40 M NaOH (Table 1). At higher concentrations, a brown precipitate was formed inside the tubes due to the formation of solid manganese dioxide ($MnO_2$). Similarly, the impact of changing the $KMnO_4$ concentrations in the range of 0.2 to 7.0 mM was investigated. The highest signal was achieved at 3.0 mM of $KMnO_4$ (Table 1).

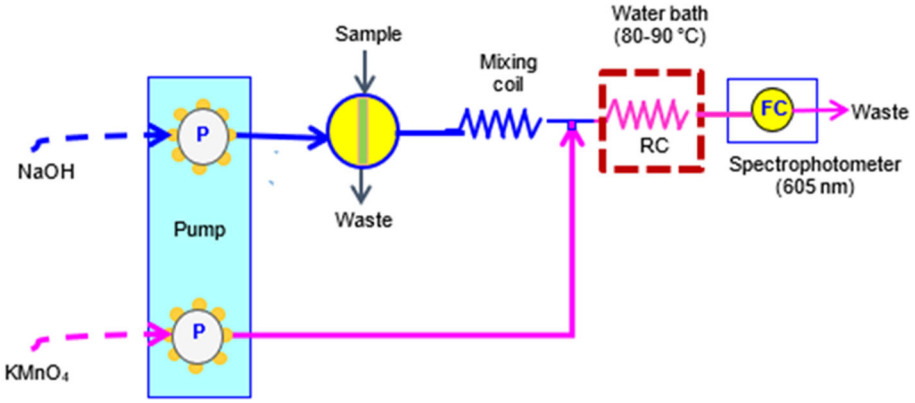


**Fig 2.** Diagrammatic representation of the suggested FIA system

**Table 1.** The effect of NaOH and $KMnO_4$ concentration on the analytical signal

| Conc. of NaOH (M) | Abs. | Conc of $KMnO_4$ (mM) | Abs. |
|---|---|---|---|
| 0 | 0.009 | 0.2 | 0.056 |
| 0.1 | 0.135 | 0.5 | 0.070 |
| 0.2 | 0.189 | 0.7 | 0.097 |
| 0.3 | 0.255 | 1.0 | 0.116 |
| 0.4 | 0.270 | 2.0 | 0.154 |
| 0.5 | 0.252 | 3.0 | 0.175 |
| 0.7 | 0.254 | 5.0 | 0.171 |
| 1.0 | 0.255 | 7.0 | 0.170 |

The FIA variables were optimized keeping the concentration of $KMnO_4$ at 3.0 mM and the injected volume at 20 µL of 50 µg/mL CLD. The flow rates of the carrier and reagent streams had a significant impact on the sensitivity and speed of analysis. The FIA manifold was constructed using micro tubes of the same diameter, which ensured that the flow rates in the two lines were always the same. The total flow rate was tested over the range of 2.0 to 6.0 mL/min, and it was observed that the highest signal was at lower pumping rates. At higher pumping rates, the signal decreased because it reached the flow cell before the reaction was completed. Different lengths of the RC were tested to increase the contact time between the reactants before measurement. Results indicated that the highest absorption signal was obtained at the lowest pumping rate and the longest RC (Fig. 3). However, the time it takes for the peak to return to the baseline before the next injection has significantly increased.

The reactants were heated before reaching the flow cell to enhance the signal and speed up the reaction. The RC was immersed in a hot water bath as shown in Fig. 2. The temperature was varied between 25 to 90 °C to speed up the reaction. The RC was made 120 cm long to maintain a stable temperature and achieve maximum signal. As a result, minor changes in the absorbance values were observed at 80 °C and beyond, as illustrated in Fig. 3. Finally, a RC length of 120 cm and a pumping rate of 2.0 mL/min at 80 °C were used as compromise conditions. At this pumping rate, one injection would take a total of 0.5 min and therefore, the sample throughput would be 120 samples per hour.

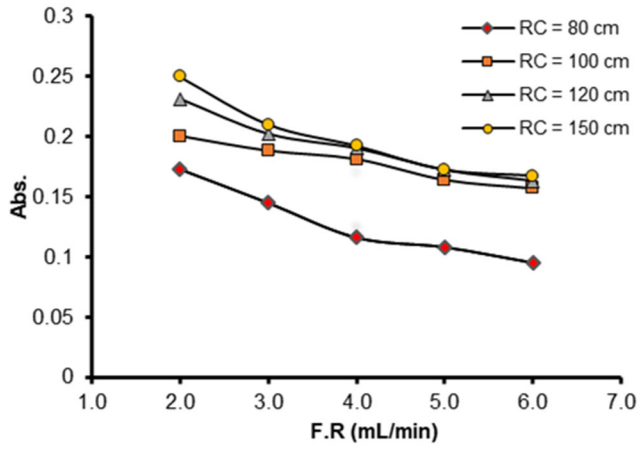


**Fig 3.** Impact of the RC length and the flow rates of reagents on the signal of 50 µg/mL CLD using the FIA manifold shown in Fig. 2

## Evaluation of the FIA Method

The calibration graphs were constructed by plotting the absorption signals against the concentration of CLD. A linear calibration curve was obtained in the range of 10 to 200 µg/mL of CLD with a correlation coefficient of 0.9979. The slope and intercept of the curve were found to be 0.0032 and 0.0180, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 1.8 and 6.0 µg/mL, respectively, using the Eq. (1) and (2);

$$\text{LOD} = 3 \times \frac{\text{SE}}{\text{slope}} \quad (1)$$

$$\text{LOQ} = 10 \times \frac{\text{SE}}{\text{slope}} \quad (2)$$

where SE is the standard error of the y-intercept. After comparing the proposed spectrophotometric method with existing methods (Table 2), it was found that the proposed method is fast, simple, has a wide linear range, and requires minimal amounts of chemicals and reagents.

To assess the precision of the method, three different concentrations within the linearity range were injected ten times for each. The average and standard deviation were calculated for each set. The results showed that the obtained relative standard deviations (%RSD) were less than 1%, indicating that the method has excellent precision. Furthermore, three different concentrations were injected at various times during the same day and on different days. The intraday and inter-day precisions were then calculated. In all instances, the relative standard deviation values were less than 5%, indicating the high precision of the FIA method.

The feasibility of the proposed FIA method for the analysis of real samples was tested by analyzing commercial pharmaceutical products containing CLD. Three commercial products available in the Jordanian market were analyzed. The results are presented in Table 3. The results showed that the claims made on the label

**Table 2.** Comparison of the proposed spectrophotometric method with other existing methods for the determination of CLD

| Idea and reagents | WL, LR, and DL* | Remarks | Ref |
|---|---|---|---|
| On-line oxidation of CLD by $KMnO_4$ using FIA | 605 nm<br>10–200 µg/mL<br>1.8 µg/mL | Fast<br>No organic solvents<br>Minimum amounts of reagents<br>Wide linear range | This study |
| Oxidation of CLD by $KIO_3$ in an acidic medium to produce $I_2$. After 45 min of heating at 60 °C, $I_2$ was extracted with cyclohexane | 520 nm<br>90–360 µg/mL | Time-consuming<br>Needs organic solvents<br>Large amounts of chemicals and reagents | [18] |
| Kinetic oxidation of CLD by $KIO_3$/KI to produce $I_3^-$. Full-color development was achieved after 40 min | 350 nm<br>1–20 µg/mL<br>0.12 µg/mL | Time-consuming<br>Large amounts of chemicals and reagents<br>Narrow linear range | [19] |
| First and second derivative after dissolving CLD in (50:50) methanol and 0.1 M NaOH | 251 nm (D1)<br>239 nm (D2)<br>60–1200 µg/mL<br>3.4–42.5 µg/mL | Poor reproducibility<br>Subjected to interferences depending on sample composition<br>Needs organic solvents | [20] |
| Ion pair complex formation between CLD and bromocresol purple followed by extraction with either dichloromethane or chloroform | 397 nm<br>8–40 µg/mL<br>3.93 µg/mL | Time-consuming<br>Large amounts of chemicals and reagents<br>Narrow linear range | [21] |
| Ion pair complex formation between CLD and bromocresol green followed by extraction with either dichloromethane or chloroform | 413 nm<br>13–32 µg/mL<br>1.30 µg/mL | Time-consuming<br>Large amounts of chemicals and reagents<br>Narrow linear range | [21] |

* WL= wavelength, LR = linear range, DL = detection limit

**Table 3.** FIA results for the analysis of CLD in commercial products (n = 5)

| Name of the commercial product | Found (µg/mL) | Taken (µg/mL) | %Recovery |
|---|---|---|---|
| Clinimycin | 20.00 | 19.79 | 98.94 |
| | 60.00 | 61.56 | 102.59 |
| | 90.00 | 89.85 | 99.84 |
| Clindamyl | 20.00 | 20.74 | 103.68 |
| | 30.00 | 31.05 | 103.49 |
| | 60.00 | 63.81 | 106.35 |
| | 90.00 | 95.04 | 105.60 |
| Clindacin | 20.00 | 19.26 | 96.28 |
| | 30.00 | 30.06 | 100.21 |
| | 60.00 | 62.66 | 104.44 |
| | 90.00 | 88.19 | 97.99 |

were accurate, with an error rate of less than 3% across all of the samples. These results provide further evidence that this methodology is suitable for use in pharmaceutical research.

## Development of the HPLC Method

Although various HPLC assays have been developed to quantify clindamycin in different matrixes,

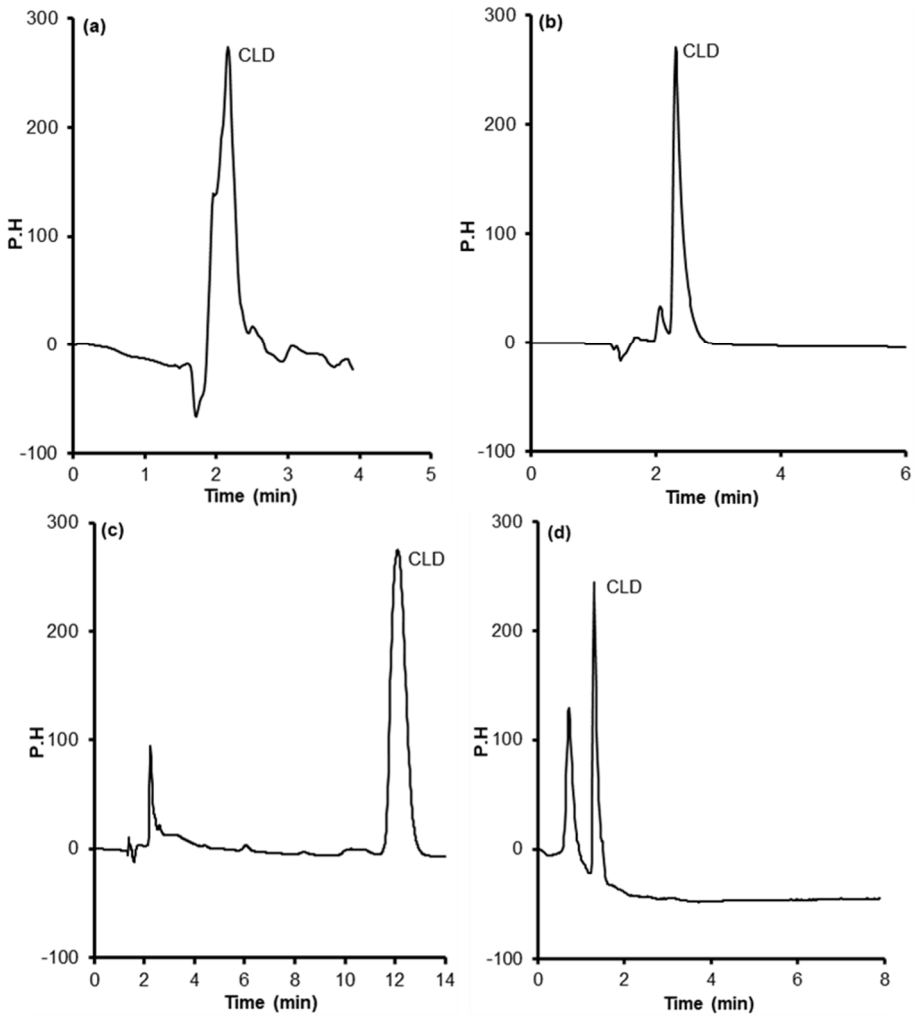


**Fig 4.** Typical chromatograms for 400 µg/mL of CLD using different mobile phases where (a) 10% phosphate buffer+ 90% MeOH, pH = 3.90, (b) 40% phosphate buffer + 30% MeOH + 30% ACN, pH = 3.45, (c) 50% phosphate buffer + 50% MeOH, 2.0 mM hexane sulfonic acid, pH = 3.22, and (d) 70% phosphate buffer + 30% ACN, pH = 6.32

these methods are not without limitations. CLD is a polar weak organic base with several active polar sites. Therefore, it is expected to elute early using the common non-polar $C_8$ or $C_{18}$ reverse phase columns. Using a $C_{18}$ column and MeOH/ACN/phosphate buffer at pH 4.5, CLD was eluted at 2.2 min with inconsistent retention times [5]. Batzias et al. [2] used tetra-*n*-butylammonium hydrogen sulfate as an ion-pairing reagent to delay the elution of CLD from the $C_{18}$ column at 40 °C. However, this method did not utilize an IS to prevent errors that may occur during sample pre-treatment and injection. Cho et al. [3] utilized cyano column, a relatively polar column compared to $C_{18}$ columns, resulting in a long total analysis time and retention times of 13.6 min for CLD and 18.6 min for the IS propranolol. Other researchers have used gradient elution to adjust the elution time of CLD and the IS [9].

In this study, several mobile phases were used as part of the optimization process. Mobile phases with different pH values and mixing ratios of the usual reversed-phase liquid chromatography solvents and modifiers such as ACN, phosphate buffer, and MeOH were tested. These mobile phases spanned a wide range of strengths, from the strongest to the weakest, in terms of drug retention within the column. Fig. 4 shows typical chromatograms obtained using different mobile phases during the optimization process. After several trials, the best result was obtained using a mixture of phosphate

buffer (50%), MeOH (35%), and ACN (15%), adjusted to a pH of 3.47 with phosphoric acid as a mobile phase. The RP-$C_8$ from Varian was used as the stationary phase. Under these conditions, multiple compounds were evaluated for their suitability as possible IS. Ciprofloxacin was finally selected as the IS. A typical chromatogram of CLD and the IS is shown in Fig. 5, which was created under the selected conditions.

## Evaluation of the HPLC Method

The method was validated following the ICH guidelines for the determination of CLD in pharmaceutical products. The accuracy, precision, linearity, robustness, specificity, system suitability, LOD, and LOQ were evaluated to validate the method. The linearity was established by injecting a series of standard solutions of CLD, each containing a constant amount of the IS solution. The freshly prepared standards were made in the range of 50 to 800 µg/mL. Fig. 6 displays the chromatogram obtained for this set of standard solutions. The peak area ratio (PAR) was calculated for each solution by dividing the area under the CLD peak by the area under the IS peak. A plot of the concentration on x-axis against the PAR on y-axis displays a linear correlation (PAR = 0.0052 C + 0.0160, r = 0.9997). The LOD was determined by successive dilution of the standard CLD solution until the signal-to-noise ratio reached approximately 3. The LOQ was determined at a

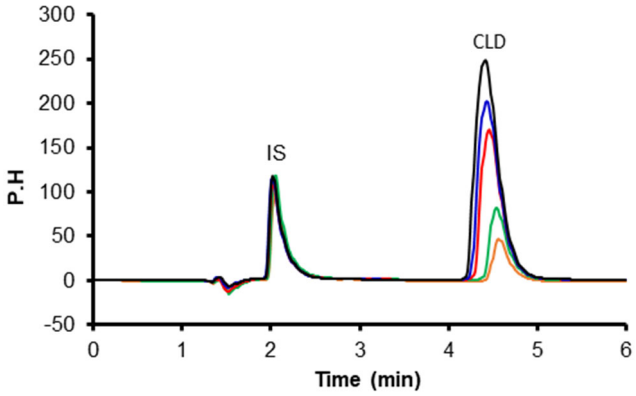


**Fig 6.** Typical chromatograms for pure CLD at different concentrations (50–400 µg/mL). using 50% phosphate buffer of 35% MeOH:15% ACN, adjusted to pH = 3.47 as mobile phase, reversed-phase $C_8$ column, 1 mL/min flow rate at 205 nm

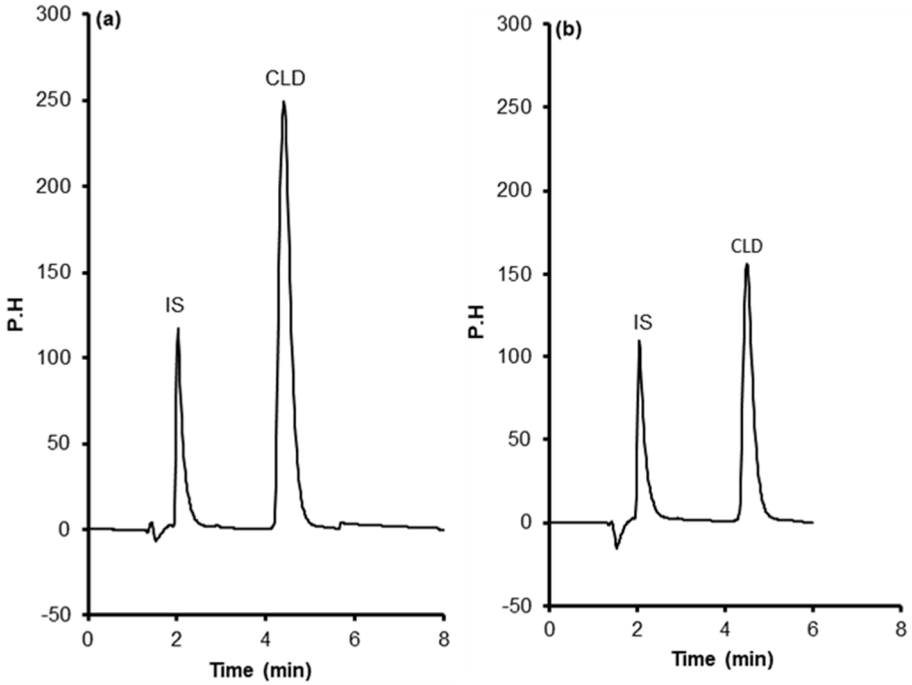


**Fig 5.** Typical chromatograms for (a) 400 µg/mL of fresh CLD and (b) 250 µg/mL of CLD in the commercial product Clindamyl using 50% phosphate buffer of 35% MeOH:15% ACN, adjusted to pH = 3.47 as mobile phase, reversed-phase $C_8$ column, 1 mL/min flow rate at 205 nm

signal-to-noise ratio of 10. The LOD and LOQ were calculated to be 12 and 40 μg/mL, respectively.

The proposed method was tested for its accuracy by injecting three different concentrations of CLD in the presence of a placebo. Each solution was injected four times. The mean percentage recovery of CLD at each level ranged from 97% to 102%, as shown in Table 4. Separate analyses were carried out at various time intervals to determine the precision and robustness of the method. For inter-day precision, the tests were conducted on different days, while for intra-day precision, all tests were conducted on the same day. The precision of the method was expressed as %RSD of the repeated measurements. As indicated in Table 4, the method is precise, with %RSD values of less than 5%.

The developed method was utilized to determine the concentration of CLD in commercial pharmaceutical formulations. Four injections were performed on average to estimate the drug content, and the results are presented in Table 5. The results were consistent with the labeled claim of the market formulations, indicating that the method used was reliable. Fig. 5(a) and (b) show the standard and sample chromatograms, respectively. It is worth noting that no interferences were observed from additives usually used in pharmaceutical formulations.

One-way ANOVA was used to compare the means of the two methods and check if there were any significant differences. ANOVA was used to compare the results of the two methods for the same samples. The obtained p-value was greater than 0.05, indicating no significant difference between the means.

## ■ CONCLUSION

The study found that FIA can be utilized to measure the concentration of CLD in various products. The results revealed that the method is reproducible with

**Table 4.** Precision and accuracy results for the proposed HPLC method for samples tested at different times

| Taken (μg/mL) | %Recovery found ± %RSD | | |
|---|---|---|---|
| Same-day precision (n = 4): | | | |
| | 0 h | 2 h | 4 h |
| 200 | 99.2 ± 2.3 | 101.0 ± 1.8 | 100.4 ± 0.6 |
| 600 | 102.1 ± 1.4 | 100.2 ± 1.1 | 97.8 ± 3.4 |
| 800 | 97.9 ± 2.7 | 100.5 ± 1.2 | 100.1 ± 0.9 |
| Different days precision (n = 4): | | | |
| | Day 1 | Day 2 | Day 3 |
| 200 | 100.1 ± 0.7 | 99.2 ± 0.8 | 100.8 ± 0.5 |
| 600 | 100.9 ± 1.4 | 102.1 ± 1.4 | 100.2 ± 1.6 |
| 800 | 100.3 ± 2.3 | 97.9 ± 3.7 | 100.1 ± 1.7 |

**Table 5.** Results of CLD analysis using HPLC on the pharmaceutical product (n = 4)

| Name of the commercial product | Labl taken (μg/mL) | Conc. found ± SD | %Recovery ± %RSD |
|---|---|---|---|
| Clinimycin | 100 | 99.5 ± 1.4 | 99.5 ± 1.2 |
| | 200 | 203.4 ± 2.6 | 101.7 ± 1.3 |
| | 400 | 400.6 ± 0.9 | 100.2 ± 0.2 |
| Clindamyl | 100 | 102.6 ± 1.6 | 102.6 ± 1.5 |
| | 200 | 192.9 ± 0.7 | 96.4 ± 0.4 |
| | 400 | 410.6 ± 2.8 | 102.7 ± 0.7 |
| | 600 | 619.4 ± 1.2 | 103.2 ± 0.2 |
| Clindacin | 100 | 101.0 ± 1.1 | 101.0 ± 0.2 |
| | 200 | 201.1 ± 0.2 | 100.5 ± 0.1 |
| | 400 | 396.6 ± 0.4 | 99.2 ± 0.1 |
| | 600 | 604.6 ± 0.7 | 100.8 ± 0.1 |

with an %RSD of less than 5% and a speed of 120 samples per hour. The developed method is fast, accurate, precise, and cost-effective as compared to the official USP chromatographic technique. It can be used to analyze CLD in different formulations without any interference. The suggested HPLC method has been utilized to determine the amount of CLD in commercial pharmaceutical formulations. The results show that the method is simple, accurate, specific, and fast. The suggested HPLC method is fast with an average analysis time of less than 5 min. It is free from any interference generally caused by the excipients commonly added to pharmaceutical products. The ANOVA analysis showed no significant differences in the results obtained by the HPLC and FIA methods.

## ■ ACKNOWLEDGMENTS

The financial support from Yarmouk University is gratefully acknowledged. The authors would like to thank MAS Pharmaceuticals Industries, and Al-Hikma Pharmaceuticals (Amman, Jordan) for providing the standard and the excipients.

## ■ CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ■ AUTHOR CONTRIBUTIONS

Idrees Faleh Al-Momani: The owner of the idea and the main supervisor of the work, formal analysis, conceptualization, validation, virtualization, and article writing. Lana Mohammad Zaid Al-Kilani: Did experimental work and methodology, samples analysis and validation of methodology. All authors accepted the final version of this manuscript.

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