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Original Article

# An ecofriendly green liquid chromatographic method for simultaneous determination of nicotinamide and clindamycin phosphate in pharmaceutical gel for acne treatment

*Fawzia Ibrahim* [a], *Asmaa Kamal El-Deen* [a,b], *Samah Abo El Abass* [a], *Kuniyoshi Shimizu* [b,*]

[a] *Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*

[b] *Department of Agro-Environmental Sciences, Graduate School of Bioresource and Bioenviromental Sciences, Kyushu University, Fukuoka 812-8581, Japan*

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ABSTRACT

A new green micellar liquid chromatographic method was developed and validated for the quantitative estimation of nicotinamide (NICO) and clindamycin phosphate (CLD) in bulk and pharmaceutical gel formulation. The analytes are well resolved in less than 6.0 minutes using micellar mobile phase consisting of 0.10M sodium dodecyl sulfate (SDS), 0.3% triethylamine, and 10% 2-propanol in 0.02M orthophosphoric acid at pH 3.0, running through an Eclipse XDB-$C_8$ column (150 mm × 4.6 mm, 5 μm particle size) with flow rate 1.0 mL/min. The effluent was monitored with diode array detection at 210 nm. The retention times of NICO and CLD were 3.8 minutes and 5.6 minutes, respectively. The method was validated according to the International Conference on Harmonisation (ICH) guidelines in terms of linearity, limit of detection, limit of quantification, accuracy, precision, robustness, and specificity to prove its reliability. Linear correlation was achieved by plotting the peak area of each drug against its concentration. It was found to be rectilinear in the ranges of 1.0–40.0 μg/mL and 0.5–15.0 μg/mL with limits of detection of 0.06 μg/mL and 0.03 μg/mL and limits of quantification of 0.19 μg/mL and 0.09 μg/mL for NICO and CLD, respectively. The method was successfully implemented for the simultaneous determination of the analytes in their bulk powder and combined gel formulation with high % recoveries. The ease of sample treatment facilitates and greatly expedites the treatment with reduced cost and improved accuracy of the procedure.



* *Corresponding author*. Department of Agro-Environmental Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.

E-mail address: shimizu@agr.kyushu-u.ac.jp (K. Shimizu).

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## 1. Introduction

Acne vulgaris is one of the most common skin disorders which mainly affect adolescents, although it may present at any age. Definitely, acne is a multifactorial chronic inflammatory disease of pilosebaceous units [1]. Recently, new therapeutic modalities and various combinations have been designed for acne treatment including benzoyl peroxide, antibiotics, retinoids, etc., as the mainstay of treatment in topical formulations [2]. Among the different available drugs for the treatment of acne, nicotinamide (NICO) and clindamycin phosphate (CLD) have been recently combined in a topical dosage form at a pharmaceutical ratio 4:1, respectively, for the treatment of mild to moderate inflammatory acne [3].

NICO (Figure 1A) [3] is chemically defined as pyridine-3-carboxamide. It is also known as nicotinic acid amide, nicotylamide, and niacinamide (vitamin B3). It is a water-soluble vitamin. Topical NICO is used in the treatment of mild to moderate inflammatory acne. CLD (Figure 1B) [3], methyl 6-amino-7-chloro-6,7,8-trideoxy-N-[(2S,4R)-1-methyl-4-propylprolyl]-1-thio-L-threo-D-galacto octopyranoside-2-(dihydrogen phosphate), is a lincosamide antibacterial with a primarily bacteriostatic action against Gram-positive aerobes and a wide range of anaerobic bacteria. CLD was found to have an activity against *Propionibacterium acnes* when used topically. Both NICO and CLD are official drugs in the United States Pharmacopoeia [4] which recommends a high performance liquid chromatography (HPLC) method for the determination of both drugs in their pure form, separately. Also, the British Pharmacopoeia [5] determined CLD by a HPLC method and NICO by a titrimetric method, both in their pure form. In addition, several methods were reported for the determination of CLD in pharmaceutical preparations, either alone or in combination with other drugs, including spectrophotometry [6–9] and HPLC [10–16]. For NICO, spectrophotometry [17,18], HPLC [19] and high performance thin layer chromatography (HPTLC) [20] were reported for its analysis in pharmaceutical preparations either alone or in combination with other drugs.

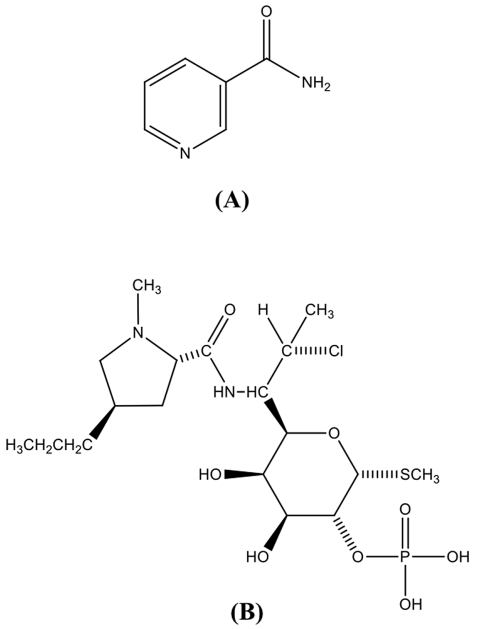


**Figure 1 – Structural formulas of (A) nicotinamide and (B) clindamycin phosphate.**

To the best of our knowledge, there is only one reported HPLC method for the simultaneous determination of NICO and CLD in coformulated preparation [21]. This method is of narrow linearity range, low column efficiency, and of lower sensitivity. Additionally, the tedious, multistep, and lengthy procedure for extraction of both drugs from their pharmaceutical formulation is one of its drawbacks. This method also consumes large quantities of organic solvents which are toxic to the analyst and the environment. So, this promoted us to develop a new rapid, inexpensive, environmentally friendly, and reliable analytical method to overcome all of these drawbacks.

Micellar liquid chromatography (MLC) has recently gained interest as an efficient alternative to conventional liquid chromatography, aiming to reduce the amount of organic solvent consumed and thus decrease the generated waste without affecting the chromatographic performance. MLC has many merits over conventional HPLC, like low environmental impact, low cost, safety, easy sample treatment, and direct on-column injection of physiological fluids [22]. Thus, MLC is exploited to allow direct injection of the sample and resolve the analytes in a short chromatographic run time.

The present study overcame the problems faced in the reported method for the simultaneous determination of NICO and CLD, since it showed excellent sensitivity with good linearity. Additionally, it is less hazardous, nontoxic, time saving, and cost-effective.

Previous trials in our laboratory were made to establish a simple and selective derivative spectrophotometric method to resolve the highly overlapping spectra of NICO and CLD, but it failed to give positive results due to the low molar absorptivity of CLD compared to NICO and also due to its lower ratio in its mixture with NICO (1:4). This added more advantages to the use of MLC method for a sensitive and selective determination of the studied drugs in their combined gel formulation.

## 2. Methods

### 2.1. Instrumentation

An Agilent 1220 Infinity LC system (G4294B configuration; Agilent Technologies, Santa Clara, CA, USA), which consisted of a dual solvent deliver system, an auto sampler, and a diode array detector (DAD), was used. An ultrasonic bath (S 100 H, Elmasonic, Singen, Germany) and a Docu pH-meter (Sartorius, Bohemia, NY, USA) were used.

### 2.2. Chemicals and reagents

All the used chemicals were of analytical reagent grade, and the solvents were of HPLC grade. NICO, CLD, SDS (90%), triethylamine (TEA), orthophosphoric acid (85%), methanol, 2-propanol, and acetonitrile (HPLC grade) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### 2.3. Pharmaceutical sample

Clinmiskin gel (Batch number CL382) labeled to contain NICO 4% and CLD 1% manufactured by Resilent Cosmeceuticals PVT.Ltd. (Maharashtra, India) was purchased from an Indian pharmacy.

### 2.4. Chromatographic conditions

An Eclipse XDB-$C_8$ column (150 mm × 4.6 mm, 5 μm particle size; Agilent Technologies) was used with a mobile phase consisting of 0.10M SDS, 10% 2-propanol, 0.3% TEA, prepared in 0.02M orthophosphoric acid at pH 3.0. The mobile phase was filtered through a 0.20-μm Millipore membrane filter (Advantec, Dublin, CA, USA) and degassed by sonication for 30 minutes before pumping with a flow rate 1.0 mL/min. The detection wavelength was monitored at 210 nm.

### 2.5. Standard solutions

Stock solutions (500 μg/mL) of NICO and CLD were prepared separately by dissolving an accurate weight of 50.0 mg of each drug in 100 mL methanol. Working solutions were prepared by further dilution of the standard solutions with the same solvent or the mobile phase. All solutions were stored in the refrigerator at 4°C and found to be stable for at least 1 week without alteration.

### 2.6. Construction of calibration graphs

Working solutions containing 1.0–40.0 μg/mL and 0.5–15.0 μg/mL of NICO and CLD, respectively, were prepared by dilution of the stock solution with the mobile phase. The solutions were well mixed and aliquots of 20 μL were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions with DAD detection set at 210 nm. The average peak area versus the final concentration of the drug in μg/mL was plotted to get the calibration graph and the corresponding regression equations were also derived.

### 2.7. Analysis of NICO/CLD laboratory-prepared mixtures

Laboratory prepared mixtures of NICO and CLD maintaining the pharmaceutical ratio of 4:1 were prepared from standard solutions in the mobile phase. The above procedure described under "Construction of the Calibration Graphs" was then applied. The percentages found were calculated by using the corresponding regression equations.

### 2.8. Analysis of the studied drugs in Clinmiskin gel by the proposed method

An appropriate weight of the gel (5.0 g) equivalent to 50 mg CLD and 200 mg NICO was directly dissolved in 100 mL micellar mobile phase to prepare a stock solution. Accurate volumes of the stock solution were diluted with the mobile phase and analyzed by the previously described procedure. The percentages found were calculated from the corresponding regression equations.

## 3. Results

### 3.1. Method development and optimization

Different conditions affecting the chromatographic separation of the cited drugs were carefully studied and incorporated into the procedure. The investigation was aimed to choose a chromatographic system with the highest number of theoretical plates and the highest resolution within a short chromatographic run time. Good chromatographic separation of the studied drugs was achieved using an Eclipse XDB-$C_8$ column (150 mm × 4.6 mm, 5 μm particle size; Agilent Technologies) with a mobile phase consisting of 0.10M SDS, 10% 2-propanol, 0.3% TEA, prepared in 0.02M orthophosphoric acid at pH 3.0, and pumped at flow rate 1.0 mL/ min with DAD detector at 210 nm (Figure 2).

### 3.2. Method validation

The proposed method was validated according to International Conference on Harmonisation (ICH) Q2R1 Guidelines [23] testing linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness, specificity, and system suitability.

#### 3.2.1. Linearity and concentration range

Linearity of the proposed method was demonstrated by plotting the peak area of each drug against its concentration in

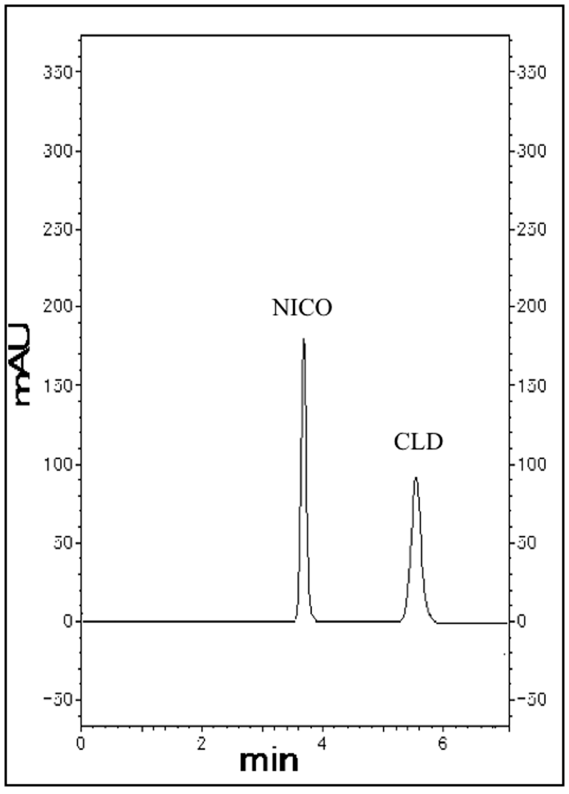


**Figure 2 – Representative chromatograms for nicotinamide (NICO; 40 μg/mL) and clindamycin phosphate (CLD) (10 μg/mL) in laboratory-prepared mixture.**

μg/mL. The concentration plots were found to be rectilinear over the ranges of 1.0–40.0 μg/mL and 0.5–15.0 μg/mL for NICO and CLD, respectively. The data was analyzed statistically [24] and the results showed high value of correlation coefficient (r) and small values of relative standard deviation as illustrated in Table 1.

#### 3.2.2. *LOQ and LOD*

LOQ and LOD were calculated according to ICH Q2R [23] recommendations using the following equations: LOQ = 10 Sa /b, LOD = 3.3 Sa /b, where Sa = standard deviation of the intercept and b = slope of the calibration curve. Table 1 illustrates the obtained results.

#### 3.2.3. *Accuracy*

To prove the accuracy of the method, the results of assay of NICO and CLD, both in pure form and in combined gel formulation, were compared with those of the comparison method [21]. Statistical analysis of the results by applying Student *t* test and variance ratio *F* test [24] revealed no significant difference between the performances of both methods (the calculated *t* and *F* values were lower than the tabulated values).

#### 3.2.4. *Precision*

Intraday precision (repeatability) and interday precision (intermediate precision) were performed through replicate analysis of three concentrations of the studied drugs in pure form on three successive times within the same day and on 3 successive days, respectively. The results of precision are summarized in Table 2.

#### 3.2.5. *Robustness*

The robustness of the HPLC method was proven by small intentional changes in the experimental parameters, including pH of the mobile phase (3.0 ± 0.1), 2-propanol concentration (10 ± 1%, v/v), concentration of SDS (0.10 ± 0.01M), and the flow rate (1.0 ± 0.1). These minor changes did not significantly affect the peak area of the studied drugs confirming the robustness of the method.

**Table 1 – Analytical performance data for the determination of the studied drugs by the proposed method.**

| Parameter | NICO | CLD |
|---|---|---|
| Linearity range (μg/mL) | 1.0–40.0 | 0.5–15.0 |
| Limit of detection (μg/mL) | 0.06 | 0.03 |
| Limit of quantification (μg/mL) | 0.19 | 0.09 |
| Correlation coefficient (r) | 0.9998 | 0.9999 |
| Intercept (a) | $4.6 \times 10^6$ | $9.7 \times 10^3$ |
| Slope (b) | $3.3 \times 10^5$ | $2.5 \times 10^5$ |
| Standard deviation of intercept (Sa) | $6.2 \times 10^3$ | $2.2 \times 10^3$ |
| Standard deviation of slope (Sb) | 935.47 | 555.17 |
| Standard deviation of residuals (Sy/x) | $3.2 \times 10^4$ | $6.9 \times 10^3$ |
| Standard deviation (SD) | 1.68 | 1.18 |
| % Relative standard deviation (%RSD) | 1.68 | 1.18 |
| % Error | 0.595 | 0.48 |

CLD = clindamycin phosphate; NICO = nicotinamide.

#### 3.2.6. *Specificity*

The proposed method specificity was tested by its ability to determine NICO and CLD in their combined gel formulation without interference from common excipients and additives, as indicated in Figure 3.

#### 3.2.7. *System suitability testing*

System suitability parameters were evaluated to prove the system performance. Parameters including *NTP*, *Rs*, and *T* were calculated and included in Table 3.

#### 3.2.8. *Pharmaceutical application*

The developed method was applied to simultaneously determine both NICO and CLD in laboratory-prepared mixture (Table 4 and Figure 2). Hence, the proposed method was successfully extended to the estimation of both NICO and CLD in their coformulated gel (Table 5). Figure 3 shows a typical chromatogram for the determination of NICO and CLD in Clinmiskin gel.

## 4. Discussion

### 4.1. *Method development and optimization*

This study aimed to develop an accurate, sensitive, and time-saving MLC method for the simultaneous estimation of NICO and CLD in their raw material and combined gel formulation. Therefore, the DAD detector response of both drugs was studied. CLD lacks a UV-absorbing chromophore and can only be detected in the low wavelength UV range. Additionally, it exists in a lower ratio in its mixture with NICO (1:4). Thus, 210 nm was chosen as the most appropriate wavelength that allows good separation of both drugs with reasonable sensitivity. Two columns were tried for performance investigation including the Eclipse XDB-$C_8$ column (150 mm × 4.6 mm, 5 μm particle size) and the Phenomenex-$C_{18}$ column (250 mm × 4.6 mm, 5 μm particle size). The Eclipse XDB-C8 column was the most suitable as it attained better separation of the studied drugs with symmetrical peaks and the highest number of theoretical plates within a reasonable analytical run time (6.0 minutes). The second column was not chosen since it gives a broad peak of NICO with low sensitivity for both drugs. Although the $C_8$ column is not a standard choice for the studied drugs, it is an inexpensive standard column found in all analytical laboratories. Hence, our method does not require the use of an expensive polar HPLC column and can be applied easily in any analytical laboratory.

To improve the performance of the chromatographic system, several modifications in the mobile phase composition were investigated. At first, different SDS concentrations in the range of (0.05–0.18M) were tested. The most appropriate concentration was 0.10M SDS since it showed a high number of theoretical plates with high sensitivity.

To enhance the column efficiency, the elution strength, and thus decrease the analysis time to an acceptable value, a small addition of a short-chain alcohol to the micellar eluent is recommended [25]. Acetonitrile, 2-propanol, and methanol were tested and 2-propanol was the organic modifier of choice

**Table 2 – Precision data for the determination of nicotinamide (NICO) and clindamycin phosphate (CLD) by the proposed method.[a]**

| Compound | Conc. (μg/mL) | Interday precision | | | Intraday precision | | |
|---|---|---|---|---|---|---|---|
| | | Mean ± SD | %RSD | %Error | Mean ± SD | %RSD | %Error |
| NICO | 4.0 | 100.04 ± 0.60 | 0.60 | 0.35 | 99.48 ± 0.58 | 0.59 | 0.34 |
| | 20.0 | 99.86 ± 0.41 | 0.42 | 0.24 | 99.42 ± 0.86 | 0.86 | 0.50 |
| | 40.0 | 99.25 ± 0.70 | 0.71 | 0.41 | 100.12 ± 1.51 | 1.51 | 0.87 |
| CLD | 1.0 | 99.27 ± 0.72 | 0.72 | 0.42 | 100.19 ± 0.81 | 0.81 | 0.47 |
| | 5.0 | 100.05 ± 0.52 | 0.52 | 0.30 | 99.51 ± 1.21 | 1.22 | 0.70 |
| | 10.0 | 99.92 ± 1.39 | 1.39 | 0.81 | 99.64 ± 0.80 | 0.80 | 0.46 |

RSD = relative standard deviation; SD = standard deviation.
[a] Each result is the average of three separate determinations.

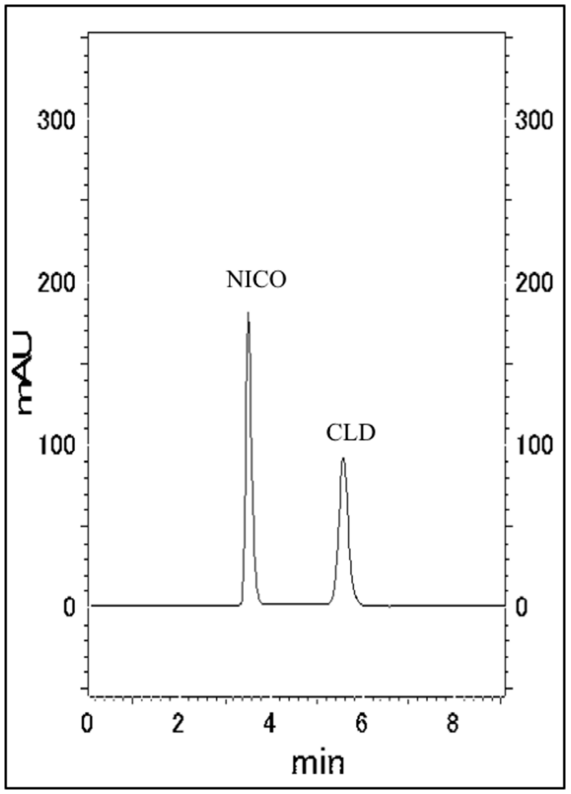


**Figure 3 – Representative chromatograms for nicotinamide (NICO) (40 μg/mL) and clindamycin phosphate (CLD) (10 μg/mL) in Clinmiskin gel.**

**Table 3 – Final system suitability test parameters for the proposed method.[a]**

| Parameter | NICO | CLD |
|---|---|---|
| No. of theoretical plates (*NTP*) | 9876 | 4343 |
| Tailing factor (*T*) | 1.11 | 1.25 |
| Resolution ($R_s$) | NICO/CLD | |
| | 12.41 | |

CLD = clindamycin phosphate; NICO = nicotinamide.
[a] Calculations were made according to United States Pharmacopoeia (USP) guidelines [4].

giving symmetrical and well-resolved peaks with the highest plate count, whereas, using methanol and acetonitrile resulted in broad peaks of both drugs. Hence, different concentrations of 2-propanol (6–15%) were tested. The suitable concentration was 10% of 2-propanol, since it could separate the studied drugs producing symmetrical peaks with high resolution and high plate count.

Concerning the effect of different pH values of the mobile phase over the range of 3–6, it was found that the most appropriate one giving well resolved peaks and the highest plate count was pH 3.0. Furthermore, this pH helps to keep the lifetime and durability of the column.

Finally, the flow rate was changed over the range of 0.5–1.0 mL/min and a flow rate of 1.0 mL/min was optimal for good separation in a short time, less than 6 minutes, with good peak symmetry.

### 4.2. *Method validation*

The proposed method was validated according to ICH Q2R1 Guidelines [23]. Linearity of the proposed method was established by the results of statistical analysis [24]. Additionally, LOQ and LOD were also calculated according to the ICH Guidelines [23].

Accuracy of the method was proved by comparing the results of assay of NICO and CLD in combined gel formulation with those of the comparison method [21]. Results are summarized in Table 5. It can be noticed that satisfactory recovery was obtained using the proposed method. Intraday precision (repeatability) and interday precision (intermediate precision) were performed. The relative standard deviations and percentage relative errors were found to be small, indicating reasonable repeatability and intermediate precision of the proposed method (Table 2).

The deliberate minor changes in the optimum chromatographic conditions did not greatly affect the peak area, *NTP*, *Rs*, and *T* of the studied drugs, confirming the robustness of the method.

Specificity of the method was confirmed by its ability to determine NICO and CLD in their combined gel formulation without interference from common excipients and additives as indicated in Figure 3.

Our developed MLC was found to be 20 times more sensitive for NICO and 10 times more sensitive for CLD than the reported HPLC method [21]. Consequently, our method is

**Table 4 – Assay results for the determination of nicotinamide (NICO) and clindamycin phosphate (CLD) in laboratory-prepared mixture by the proposed method.[a]**

| | Proposed method | | | | | | Comparison method [21] | |
|---|---|---|---|---|---|---|---|---|
| | Amount taken (μg/mL) | | Amount found (μg/mL) | | % Found | | % Found | |
| | NICO | CLD | NICO | CLD | NICO | CLD | NICO | CLD |
| | 4.0 | 1.0 | 3.99 | 1.0 | 99.74 | 100.31 | 100.16 | 99.85 |
| | 20.0 | 5.0 | 20.03 | 4.94 | 100.13 | 98.72 | 99.81 | 98.82 |
| | 40.0 | 10.0 | 39.62 | 9.85 | 99.04 | 98.53 | 99.31 | 99.11 |
| Mean | | | | | 99.64 | 99.19 | 99.77 | 99.26 |
| ± SD | | | | | 0.552 | 0.997 | 0.417 | 0.531 |
| *t* | | | | | 0.31 | 0.11 | | |
| *F* | | | | | 1.67 | 3.39 | | |

SD = standard deviation.
[a] Each result is the average of three separate determinations. The tabulated *t* and *F* values are 2.776 and 19.00, respectively, at $p = 0.05$ [24].

**Table 5 – Assay results for the determination of nicotinamide (NICO) and clindamycin phosphate (CLD) in Clinmiskin gel.[a]**

| Preparation | Proposed method | | | | | | Comparison method [21] | |
|---|---|---|---|---|---|---|---|---|
| | Amount taken (μg/mL) | | Amount found (μg/mL) | | % Found | | % Found | |
| | NICO | CLD | NICO | CLD | NICO | CLD | NICO | CLD |
| Clinmiskin gel 15 gm | 4.0 | 1.0 | 3.96 | 0.99 | 99.02 | 99.11 | 101.22 | 99.68 |
| (4% NICO + 1% CLD) | 20.0 | 5.0 | 19.91 | 4.94 | 99.54 | 98.85 | 98.78 | 100.32 |
| | 40.0 | 10.0 | 40.41 | 10.31 | 101.02 | 100.31 | 100.41 | 99.89 |
| Mean | | | | | 99.86 | 99.42 | 100.14 | 99.96 |
| ± SD | | | | | 1.038 | 0.776 | 1.24 | 0.33 |
| *t* | | | | | 1.149 | 1.209 | | |
| *F* | | | | | 3.94 | 4.48 | | |

SD = standard deviation.
[a] Each result is the average of three separate determinations. The tabulated *t* and *F* values are 2.776 and 19.00 at $p = 0.05$, respectively [24].

simple, with no need for the tedious, multistep, and lengthy procedure for extraction of both drugs from their pharmaceutical gel formulation. It is also less hazardous, nontoxic, and cost-effective.

### 4.3. *Pharmaceutical application*

The coformulated gel containing NICO and CLD could be successfully analyzed by the developed method. The results were found to be accurate and proved the applicability of the method for quality control (Table 5).

## 5. Conclusion

The present study represents the first ecofriendly MLC method for the simultaneous determination of NICO and CLD in their bulk and combined gel formulation. Micellar liquid chromatographic determination of NICO and CLD in their combined gel formulation has the following advantages: speed, direct sample injection without sample pretreatment (it avoids extraction losses during the evaporation and reconstitution steps), enhanced reproducibility, low environmental impact, and safety. Wide linearity ranges were obtained; 1.0–40.0 μg/mL and 0.5–15.0 μg/mL for NICO and CLD, respectively, with lower detection limits of 0.06 μg/mL and 0.03 μg/mL, respectively. The good validation criteria of the proposed method allow its use in quality control laboratories.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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