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## Review Paper

# A Review of Analytical Methods for the Determination of Clindamycin in Pharmaceutical Products and Environmental Samples

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## ABSTRACT

Antibiotics represent one of the most consequential classes of pharmaceuticals from an environmental standpoint, with growing evidence of their adverse effects on aquatic and soil ecosystems. Clindamycin (CLN), a lincosamide antibiotic that inhibits bacterial protein synthesis by targeting the 50S ribosomal subunit, is active against Gram-positive aerobes and a broad range of anaerobes. It is also employed in the management of acne vulgaris, toxoplasmosis, malaria, Pneumocystis pneumonia, and babesiosis. The continuous discharge of CLN and its metabolites into water bodies—through incomplete metabolism, improper drug disposal, and inadequate wastewater treatment—has prompted growing concern about its environmental fate and ecotoxicological implications. Reliable analytical methods are therefore indispensable for its accurate detection and quantification across complex matrices. This review systematically examines the principal analytical techniques reported for CLN determination in pharmaceutical dosage forms and environmental samples. The methods covered include high-performance liquid chromatography (HPLC) with UV and mass spectrometric detection, spectrophotometric and kinetic spectrophotometric procedures, electrochemical techniques such as stripping voltammetry and potentiometry, micellar electrokinetic chromatography (MEKC), capillary electrophoresis (CE), and chemiluminescence assays. For each approach, the working principle, matrix applicability, sensitivity, and key validation parameters are discussed. The review aims to serve as a concise reference for analysts selecting appropriate methods for routine quality control, stability studies, or environmental monitoring of this clinically important antibiotic.

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## INTRODUCTION

The rapid expansion of pharmaceutical manufacturing over the past five decades has created a class of environmental contaminants that regulatory frameworks were not originally designed to address. Pharmaceuticals and personal care products (PPCPs) enter the environment through multiple routes—excretion of unchanged drug and active metabolites, hospital and household effluents, and the disposal of unused medicines—accumulating in surface water, groundwater, and sediments at concentrations typically in the nanogram-to-microgram per litre range.<sup>1,2</sup>

Although individual environmental concentrations appear low, the continuous, pseudo-persistent nature of pharmaceutical input means that aquatic organisms face chronic exposure rather than episodic contact. Among the major therapeutic classes, antibiotics attract particular concern because their biological activity does not simply diminish upon environmental entry; instead, sub-inhibitory concentrations can selectively pressure microbial communities and enrich for antimicrobial resistance genes (ARGs), which are now recognized as emerging environmental pollutants in their own right.<sup>3,4</sup>

Globally, the three pharmaceutical categories most widely detected in the environment are anti-inflammatories, antibiotics, and analgesics. Veterinary use compounds this burden: antibiotics administered in livestock production are frequently excreted largely unchanged and reach soil and aquatic compartments via manure application and agricultural runoff.<sup>5-9</sup>

Within this broad antibiotic landscape, certain drugs command disproportionate attention based on their physicochemical properties, environmental stability, and ecotoxicological profile. Clindamycin, a semisynthetic derivative of lincomycin produced by *Streptomyces*

*lincolnensis*, is one such compound. Its high water solubility, moderate lipophilicity ( $\log P \approx 2.16$ ), and limited photodegradation in neutral conditions facilitate its persistence in aquatic systems. CLN has been detected in hospital effluents, municipal wastewater treatment plant influents and effluents, and in surface water bodies across multiple continents, underscoring the inadequacy of conventional treatment processes in its removal.<sup>10-14</sup>

Clindamycin inhibits bacterial protein synthesis by binding irreversibly to the peptidyl transferase centre of the 50S ribosomal subunit, preventing peptide bond formation and terminating chain elongation. This mechanism confers activity against Gram-positive aerobic bacteria (e.g., *Staphylococcus aureus*, *Streptococcus* spp.), anaerobic organisms (e.g., *Bacteroides fragilis*, *Clostridium perfringens*), and several protozoan pathogens. Clinically, CLN is used in the treatment of skin and soft-tissue infections, intra-abdominal sepsis, aspiration pneumonia, osteomyelitis, acne vulgaris, and as prophylaxis or treatment in toxoplasmosis, malaria, *Pneumocystis jirovecii* pneumonia, and babesiosis.<sup>15,16</sup>

From an environmental perspective, the antimicrobial activity that makes CLN therapeutically valuable also makes it ecotoxicologically relevant. Studies have demonstrated that CLN-containing waters exert selective pressure on environmental microbiota, alter microbial community structure, and co-select for resistance determinants associated with other antibiotic classes through cross-resistance or co-resistance mechanisms.<sup>17-19</sup>

The spread of ARGs in environmental compartments is now considered one of the defining public health challenges of the coming decades. Even though wastewater treatment measurably reduces the abundance of ARGs compared with untreated sewage, residual



resistance determinants in treated effluents continue to be released into receiving water bodies, from where they can disseminate via horizontal gene transfer—particularly through conjugative plasmids—across geographically distant microbial communities.<sup>20–24</sup>

Robust analytical methods are therefore essential at two levels: (i) in pharmaceutical quality control, to ensure accurate potency in finished dosage forms and to characterise degradation behaviour, and (ii) in environmental monitoring, to detect and quantify CLN at trace levels across varied and complex matrices. The physicochemical profile of CLN—specifically its weak UV chromophore (maximum absorbance near 195 nm) and the absence of a strong fluorophore—complicates detection by conventional photometric approaches

and has driven interest in alternative or hyphenated techniques.<sup>25,26</sup>

This review compiles and critically appraises the analytical literature on CLN determination in pharmaceutical products and environmental samples published through 2024. The methods addressed span HPLC-UV, HPLC-MS and HPLC-MS/MS, spectrophotometry, kinetic spectrophotometry, titrimetry, stripping voltammetry, potentiometry, capillary electrophoresis, micellar electrokinetic chromatography, and chemiluminescence. Table 1 summarises the key physicochemical properties of CLN that underpin method selection; Tables 2 and 3 provide comparative performance data for chromatographic and non-chromatographic methods, respectively.

**TABLE 1: PHYSICOCHEMICAL PROPERTIES OF CLINDAMYCIN**

PROPERTY	DETAILS
IUPAC Name	Methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-L-threo- $\alpha$ -D-galactooctopyranoside
Molecular Formula	C <sub>18</sub> H <sub>33</sub> ClN <sub>2</sub> O <sub>5</sub> S
Molecular Weight	424.98 g/mol (hydrochloride: 461.44 g/mol)
CAS Number	18323-44-9 (base); 21462-39-5 (HCl salt)
Appearance	White to off-white crystalline powder
Solubility	Freely soluble in water; sparingly soluble in ethanol; practically insoluble in organic solvents
pKa	7.6
log P	2.16
UV Absorption (max)	195 nm (weak absorption; lacks a strong chromophore)
Protein Binding	~93%
Half-life	2–3 hours (extended in severe hepatic impairment)
Mechanism of Action	Inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit
Antimicrobial Spectrum	Gram-positive aerobes, anaerobes, and some protozoal pathogens (e.g., Toxoplasma, Plasmodium)

CLN-P = clindamycin phosphate; CLN-HCl = clindamycin hydrochloride; NR = not reported; CPH = clindamycin palmitate hydrochloride



## **ANALYTICAL METHODS FOR DETERMINATION OF CLINDAMYCIN**

### **High-Performance Liquid Chromatography (HPLC)**

HPLC remains the workhorse technique for CLN determination, largely because reversed-phase systems using C18 stationary phases afford excellent selectivity for the drug across diverse matrices, and the method is amenable to coupling with UV, fluorescence, and mass spectrometric detectors. The weak UV absorption of CLN at 195 nm is a practical limitation for sensitivity, but short-wavelength UV detection is nonetheless widely employed for pharmaceutical-grade samples where concentrations are high and matrix interference is manageable.<sup>27</sup>

Vella and colleagues described a straightforward reversed-phase HPLC-UV method for CLN determination in human plasma. Separation was performed on a 250 × 4.6 mm C18 column (5 μm particle size) using an isocratic mobile phase of acetonitrile and 0.02 M disodium hydrogen phosphate buffer (pH 2.9) in a 29:71 v/v ratio, with UV detection at 195 nm. The approach achieved a lower limit of quantification suitable for pharmacokinetic monitoring of plasma samples.<sup>28</sup>

For veterinary applications, Batzias et al. developed and validated an HPLC-UV method capable of quantifying CLN in dog blood serum at concentrations as low as 80 ng/mL. Sample preparation involved protein precipitation with acetonitrile followed by liquid-liquid extraction with dichloromethane. Chromatographic separation on a RP-C18 column used tetra-n-butylammonium hydrogen sulfate as an ion-pairing agent, which improved retention and peak shape for this basic analyte. The assay was validated over a linear concentration range that covered clinically relevant serum levels in companion animals.<sup>29</sup>

Clindamycin phosphate (CLN-P), the water-soluble prodrug used in injectable and topical formulations, has been the focus of several HPLC methods targeting gel and vaginal formulations. Stankovic et al. described a RP-HPLC procedure for CLN-P in vaginal gels, demonstrating specificity for the prodrug against its degradation products. Modi and Shah subsequently developed a stability-indicating RP-HPLC method for the simultaneous determination of CLN-P, adapalene, methylparaben, and phenoxyethanol in topical acne gel formulations. Chromatographic resolution was achieved on an XBridge C18 column; CLN-P and the preservatives were monitored at 210 nm, while adapalene was detected at 321 nm. A key contribution of this work was the optimised gel extraction procedure, which ensured complete recovery of CLN-P from the polymer matrix without degradation of the analyte.<sup>30,31</sup>

For oral liquid preparations, Ciavarella et al. reported a validated isocratic RP-HPLC assay for clindamycin palmitate hydrochloride (CPH) and its oral solution products. The method met USP Category I validation requirements, demonstrating linearity greater than 0.999 over three consecutive days. Analysis of two commercial CPH products yielded potency estimates greater than 98%, and the approach showed higher chromatographic efficiency and reduced organic solvent consumption compared with the then-current USP compendial procedure.<sup>32</sup>

Corbett described the development of RP-HPLC methods for a series of therapeutic agents including CLN, providing a methodological template for assay development across structurally diverse lincosamide antibiotics.<sup>33</sup>

### **Hyphenated Liquid Chromatography–Mass Spectrometry Methods**

For trace-level determinations in biological and environmental samples, HPLC coupled to mass



spectrometry offers substantial advantages in sensitivity and selectivity over UV detection. Two landmark contributions illustrate the capabilities of this approach for CLN.

Yu and colleagues developed an LC-ESI-MS/MS method for CLN in human plasma, validated primarily for bioequivalence applications. The mobile phase comprised 80% acetonitrile containing 0.01% trifluoroacetic acid, with verapamil used as an internal standard. Electrospray ionization with tandem mass spectrometry detection in selected reaction monitoring (SRM) mode afforded a chromatographic run time of approximately two minutes and a detection limit of 1.3 pg/mL—among the lowest reported for CLN by any technique. The method was applied successfully to plasma samples collected from healthy volunteers over twelve hours following a single 300 mg oral dose.<sup>34</sup>

Martens-Lobenhoffer and Banditt reported an HPLC-APCI-MS method specifically designed to quantify CLN in both human serum and bone tissue, a clinically important matrix for the management of osteomyelitis and peri-prosthetic joint infections. Lincomycin was employed as the internal standard. Serum samples underwent protein precipitation with acetonitrile alone, while bone tissue required additional mechanical homogenisation. Chromatographic elution used a water–acetonitrile mobile phase (60:40 v/v) containing 0.02% trifluoroacetic acid. The atmospheric pressure chemical ionization source provided robust ionisation across both matrix types, yielding concentrations that correlated well with concurrent microbiological assay data.<sup>35</sup>

### **Spectrophotometric Methods**

Spectrophotometric methods, while less sensitive than chromatographic approaches, retain an important practical role in pharmaceutical quality control because of their simplicity, low cost, and

freedom from complex instrumentation. El-Yazbi and Blaih reported two complementary techniques for CLN hydrochloride determination in capsule and pure-drug form. The spectrophotometric procedure exploited oxidation of the drug's thioether sulfur atom by potassium iodate in acidic medium; the liberated iodine was extracted and quantified photometrically. The titrimetric approach involved direct titration with N-bromosuccinimide to the same oxidative endpoint. Both techniques yielded relative standard deviations below 1%, confirming acceptable precision for routine quality control.<sup>36</sup>

Recognizing the environmental and occupational hazards associated with organic solvents in conventional spectrophotometry, Affas and Sakur later introduced a validated green, solvent-free kinetic spectrophotometric method for CLN hydrochloride. The procedure exploited the time-dependent colour development of an oxidative reaction, measured at a fixed time point under optimised conditions. Analytical parameters such as temperature, reagent concentration, and reaction time were systematically investigated. Results were in close agreement with those obtained by the reference compendial method, and the approach was applied to both pure drug substance and capsule formulations.<sup>37</sup>

### **Electrochemical Methods**

Electrochemical techniques offer attractive analytical characteristics for CLN: high sensitivity, low sample volume requirements, and straightforward miniaturisation. The thioether group and tertiary amine of CLN are electrochemically active, providing a basis for both oxidative and reductive detection strategies. Norouzi and colleagues exploited fast Fourier transform continuous cyclic voltammetry (FFT-CCV) at a gold microelectrode in a flow injection analysis (FIA) system to achieve sub-nanomolar CLN detection. The method required no prior



deoxygenation of the sample—a practical advantage over classical polarographic techniques—and achieved a detection limit of 1.3 pg/mL with a quantitation limit of 4 pg/mL. The linear dynamic range was extraordinarily wide, spanning approximately four orders of magnitude (4 to 42,498,400 pg/mL), making the approach applicable both to trace environmental samples and to pharmaceutical formulations without dilution.<sup>38</sup>

A complementary approach was described by Habib et al., who employed adsorption stripping voltammetry at a carbon paste electrode to determine CLN in pharmaceutical dosage forms and biological samples. Carbon paste electrodes are inexpensive, renewable, and easily modified; their large usable potential window and low background current are advantageous for analytes like CLN that require anodic oxidation. The method provided selective and sensitive quantification across the concentration ranges relevant to both quality control and bioanalytical applications.<sup>39</sup>

Potentiometric determination using ion-selective electrode (ISE) technology represents a further electrochemical option, particularly suited to high-throughput pharmaceutical manufacturing environments where speed and simplicity are paramount. Mery et al. described the development of ISE sensors for CLN, characterising their Nernstian response behaviour and validating selectivity against common excipients and formulation components.<sup>40</sup>

### Capillary Electrophoresis and Micellar Electrokinetic Chromatography

Capillary zone electrophoresis (CZE) and related electrokinetic methods have attracted interest as alternatives to HPLC for CLN analysis, especially where minimal sample preparation and rapid analysis are priorities. Flurer and Wolnik demonstrated that chemical profiling by CE could

differentiate CLN sourced from different manufacturers and lots—an application of forensic and regulatory significance in verifying drug authenticity and origin.<sup>41</sup>

Dehouck and colleagues developed and validated a micellar electrokinetic chromatography (MEKC) method for CLN using a mixed micellar system. Separation conditions were optimised by central composite experimental design, identifying capillary temperature and surfactant concentration as critical parameters. At the optimised conditions (15°C capillary temperature), the method produced excellent linearity and within-day repeatability. The limits of detection and quantification were 0.05% and 0.15%, respectively, relative to a working concentration of 2.5 mg/mL CLN. Robustness testing confirmed method performance over deliberate small variations in key parameters.<sup>42</sup>

### Chemiluminescence Methods

Chemiluminescence (CL) detection offers exceptional sensitivity and very low background, because it does not depend on an external light source. Shao and co-workers developed a rapid CL method for CLN in capsules based on the drug's inhibitory effect on the myoglobin-catalysed luminol CL reaction. CLN quenches the CL signal in a concentration-dependent manner, and the decrease in CL intensity was linear with the logarithm of CLN concentration. The method had a standard deviation of less than 3.0% (n = 5) and was applied directly to capsule samples with minimal sample preparation.<sup>43</sup>

### Environmental and Multi-Residue Analytical Methods

Detection of CLN in environmental matrices introduces additional challenges: concentrations in surface water are typically in the low ng/L range, matrix complexity is high, and structurally similar analogues (e.g., lincomycin) may co-occur.



Seifrtová et al. provided a comprehensive overview of analytical methodologies for antibiotic determination in environmental waters, noting that LC-MS/MS in negative or positive electrospray ionization mode, often with solid-phase extraction (SPE) preconcentration, represents the current gold standard for trace antibiotic monitoring.<sup>44</sup>

For CLN specifically, the ionization mass spectrometry approaches described above for biological matrices (APCI-MS, ESI-MS/MS) are directly transferable to environmental applications with appropriate preconcentration steps. The high sensitivity of MS/MS detection—routinely achieving sub-nanogram-per-litre quantification

limits after SPE—is particularly valuable for surface water monitoring, where CLN concentrations rarely exceed 100 ng/L even in heavily impacted catchments.

Electrospray ionization combined with fast Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) and other high-resolution MS platforms have also been explored for untargeted environmental screening of antibiotic residues, including CLN, in wastewater effluents. These approaches, while not yet routine in regulatory monitoring, hold promise for identifying transformation products and conjugates of CLN that conventional targeted methods would miss.<sup>25</sup>

**TABLE 2: SUMMARY OF HPLC AND LC-MS METHODS FOR CLINDAMYCIN DETERMINATION**

Matrix	Column / Stationary Phase	Mobile Phase	Detection	LOD / LOQ	Reference
Human plasma	RP-C18 (250×4.6 mm, 5 µm)	ACN : 0.02 M Na <sub>2</sub> HPO <sub>4</sub> buffer pH 2.9 (29:71 v/v)	UV, 195 nm	80 ng/mL	Vella et al.
Dog blood serum	RP-C18 analytical column	ACN extraction; TBA-HSO <sub>4</sub> counter-ion	UV	80 ng/mL	Batzias et al.
Vaginal gel	RP-C18	Phosphate buffer : ACN gradient	UV, 210 nm	NR	Stankovic et al.
Topical gel (CLN-P + adapalene)	XBridge C18	Gradient (aqueous buffer : ACN)	UV, 210 nm (CLN-P); 321 nm (adapalene)	NR	Modi & Shah
Oral solution (CPH)	RP-C18, isocratic	Aqueous buffer : organic modifier	UV	Linearity >0.999	Ciavarella et al.
Human serum / bone tissue	RP-C18	Water 60% : ACN 40% + 0.02% TFA	APCI-MS	Sub-µg/g (bone)	Martens-Lobenhoffer et al.
Human plasma (bioequivalence)	RP-C18	80% ACN + 0.01% TFA	ESI-MS/MS (~2 min run)	1.3 pg/mL (LOD)	Yu et al.

ACN = acetonitrile; TFA = trifluoroacetic acid; APCI = atmospheric pressure chemical ionisation; ESI = electrospray ionisation; NR = not reported; CLN-P = clindamycin phosphate; CPH = clindamycin palmitate hydrochloride; LOD = limit of detection

**TABLE 3: SUMMARY OF NON-CHROMATOGRAPHIC METHODS FOR CLINDAMYCIN DETERMINATION**

Technique	Principle / Reagent	Matrix	Key Performance	Reference
Spectrophotometry (oxidimetric)	KIO <sub>3</sub> -mediated sulfur oxidation → iodine extraction	Pure drug; capsules	RSD < 1%; linear range validated	El-Yazbi & Blaih
Titrimetry	Direct N-bromosuccinimide titration	Capsules	RSD < 1%	El-Yazbi & Blaih
Kinetic spectrophotometry	Reaction-rate measurement (green, solvent-free)	Pure drug; capsules	Results consistent with reference method	Affas & Sakur
Micellar electrokinetic chromatography (MEKC)	Mixed micellar system; CCD-optimised; 15°C capillary temp.	Pharmaceutical solution (2.5 mg/mL)	LOD 0.05%; LOQ 0.15%; robust	Dehouck et al.
Capillary electrophoresis	Chemical profiling for drug origin	Pharmaceutical products	Selective profiling	Flurer & Wolnik
Chemiluminescence	Myoglobin-luminol CL inhibition assay	Capsules	SD < 3.0% (n=5); linear log[CLN]	Shao et al.
Fast Fourier adsorptive stripping voltammetry (FFASV)	Cyclic voltammetry at Au microelectrode; flow injection	Pure & pharmaceutical samples	LOD 1.3 pg/mL; range 4–42,498,400 pg/mL	Norouzi et al.
Adsorption stripping voltammetry	Carbon paste electrode	Dosage forms; biological samples	Sensitive, selective	Habib et al.
Potentiometry	Ion-selective electrode	Pharmaceutical formulations	Selective response	Merey et al.

*CL = chemiluminescence; MEKC = micellar electrokinetic chromatography; CE = capillary electrophoresis; RSD = relative standard deviation; LOD = limit of detection; LOQ = limit of quantification*

## CONCLUSION

The body of analytical literature reviewed here reflects the sustained demand for reliable CLN assays driven by its expanding therapeutic applications and growing environmental footprint. No single technique is universally optimal: the choice depends on the matrix, the concentration range of interest, available instrumentation, and whether the goal is regulatory compliance testing,

pharmacokinetic characterisation, or environmental surveillance.

For pharmaceutical quality control of finished dosage forms—tablets, capsules, gels, oral solutions—RP-HPLC with UV detection at 195–210 nm remains the most widely adopted approach, and several validated stability-indicating methods now allow simultaneous quantification of CLN alongside co-formulated actives and preservatives. Where regulatory specificity requirements are stringent, LC-MS/MS



or HPLC-APCI-MS provide unambiguous identity confirmation alongside quantification.

For bioanalytical work (pharmacokinetics, bioequivalence, drug monitoring in unusual matrices such as bone tissue), mass spectrometric detection is increasingly indispensable, with ESI-MS/MS achieving picogram-per-millilitre sensitivity that far exceeds what UV-based systems can provide. The FFASV-FIA method of Norouzi et al. stands out as a notable exception: its sub-nanomolar detection limit at a gold microelectrode, combined with a linear range spanning more than seven orders of magnitude and negligible deoxygenation requirements, makes it one of the most sensitive electrochemical approaches described for any antibiotic.

For low-cost, routine testing in resource-limited pharmaceutical settings, the kinetic spectrophotometric and titrimetric methods remain viable alternatives, provided that matrix complexity is low and the required sensitivity is in the microgram-per-millilitre range.

Looking forward, several gaps in the CLN analytical literature deserve attention. Environmental monitoring at clinically relevant resistance-selection thresholds (typically sub- $\mu\text{g/L}$ ) demands LC-MS/MS with rigorous SPE preconcentration protocols, and method standardisation across laboratories remains poor. The characterisation of CLN transformation products in water and sediment—chlorinated, hydroxylated, and conjugated species—is still largely incomplete, limiting our understanding of the true environmental risk profile of this antibiotic. High-resolution mass spectrometry-based screening platforms are positioned to address these gaps as instrument accessibility increases. Green analytical chemistry principles—minimising organic solvent use, reducing sample volume, and avoiding toxic derivatisation reagents—should be central to the design of next-generation CLN methods, as demonstrated by the

solvent-free kinetic spectrophotometric approach already reported for this analyte.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in connection with the material presented in this manuscript.

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