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Research Paper

A Research on Method Development and Validation of Chloramphenicol Dosage Form by UV-Visible Spectrophotometry: A Comparative Study of API and Marketed Formulations

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ABSTRACT

A simple, rapid, accurate, precise, and economical UV-Visible spectrophotometric method has been developed and validated for the quantitative estimation of chloramphenicol in bulk drug (API) and three pharmaceutical dosage forms — capsules (Chloranicol 250), injection (Lykacetin), and eye drops (CholraCare). The method is based on Beer–Lambert's law; absorbance was measured at 278 nm using methanol as solvent. Calibration curves were constructed over the concentration range of 2–10 µg/mL, demonstrating excellent linearity (R^2 0.9942). Method validation was performed in accordance with ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and repeatability. Recovery studies yielded values in the range of 100.0–100.8%, indicating high accuracy. Precision (%RSD) for all samples was below 2% (0.36–0.38%), confirming excellent reproducibility. LOD and LOQ values ranged from 0.070–0.075 ppm and 0.213–0.228 ppm, respectively, demonstrating adequate sensitivity. Percentage purity of all formulations was within the accepted pharmacopoeial limits (95.16–96.87%). The proposed method is suitable for routine quality control analysis of chloramphenicol in pharmaceutical preparations

INTRODUCTION

Chloramphenicol (CAP) is a broad-spectrum antibiotic first isolated in 1947 from Streptomyces venezuelae and subsequently synthesized chemically, marking it as one of the earliest antibiotics to be mass-produced by total chemical synthesis [1]. Its molecular formula is C11H12Cl2N2O5,

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with a molecular weight of 323.13 g/mol, and it acts by inhibiting bacterial protein synthesis through binding to the 50S ribosomal subunit [2].

Despite its well-known myelosuppressive side effects, chloramphenicol remains clinically relevant for the treatment of multidrug-resistant typhoid fever, bacterial meningitis, rickettsial infections, and ocular infections such as conjunctivitis, where topical application minimizes systemic toxicity [3,4]. It is available in multiple dosage forms including capsules, injections, eye drops, and ointments.

Accurate and reliable analytical methods for the estimation of chloramphenicol are essential for quality control of pharmaceutical formulations and for monitoring drug content in clinical and environmental matrices. Several analytical techniques have been reported for its determination, including HPLC [7,9], GC-MS, LC-MS/MS [7], electrochemical methods [19], and spectrophotometry [1,2,3,4,5,6]. Among these, UV-Visible spectrophotometry is the most widely adopted for routine analysis in pharmaceutical laboratories owing to its simplicity, cost-effectiveness, minimal sample preparation, and the intrinsic UV-absorbing chromophore present in the chloramphenicol molecule — the aromatic nitrobenzene ring system that absorbs strongly at approximately 278 nm [2,3].

Various spectrophotometric approaches have been reported for chloramphenicol determination, including direct UV measurement [2,3], oxidative complexation [1], diazotization-coupling reactions [6], area-under-curve (AUC) methods [3], and derivative spectroscopy [4,5]. However, there remains a need for a validated, simple direct UV method that can simultaneously compare API and multiple marketed dosage forms under identical conditions, enabling a meaningful quality comparison. The present study describes the development and ICH Q2(R1)-compliant validation of a direct UV method at 278 nm for the simultaneous estimation of chloramphenicol in API, capsules, injection, and eye drops using methanol as solvent.

MATERIAL AND METHOD

Drug Profile and Chemicals

Chloramphenicol API (purity 99%) was obtained from a certified supplier. Marketed formulations studied were: Chloranicol 250 (Zeelab Pharmacy, 250 mg capsules), Lykacetin (Alivira, 3 g/vial injection — chloramphenicol sodium succinate), and CholraCare (Advocare, 0.5% w/v eye drops). Methanol (LR grade) and sterile water were used as solvents. All chemicals were of analytical reagent grade.

Instrumentation

A UV-Visible spectrophotometer equipped with a 1 cm matched quartz cuvette was used for all absorbance measurements. Supporting equipment included an analytical balance, a pH meter, volumetric glassware (10 mL, 100 mL), and a sonicator.

Selection of Analytical Wavelength

A standard solution of chloramphenicol (10 µg/mL) in methanol was scanned between 200 and 400 nm. The wavelength of maximum absorption (max) was found at 278 nm, consistent with values reported in the literature [2,3]. This wavelength was selected for all subsequent measurements.

Preparation of Standard Stock Solutions

Stock Solution A (1000 µg/mL): 100 mg of chloramphenicol API was accurately weighed, dissolved in 40 mL of methanol in a 100 mL volumetric flask, and made up to volume with methanol.

Stock Solution B (100 µg/mL): 10 mL of Stock Solution A was pipetted into a 100 mL volumetric

flask and diluted to volume with methanol. Working standard solutions of 2, 4, 6, 8, and 10 µg/mL were prepared by appropriate dilution of Stock Solution B.

Preparation of Sample Solutions

Capsules (Chloranicol 250): Twenty capsules were weighed and finely powdered. A quantity equivalent to 100 mg of chloramphenicol was dissolved in methanol, sonicated for 5 minutes, and filtered. The filtrate was diluted stepwise to obtain a working concentration of 6 µg/mL.

Injection (Lykacetin): A volume corresponding to 1000 µg/mL was pipetted into a 100 mL volumetric flask and diluted to volume with methanol (primary stock). A secondary stock of 100 µg/mL was prepared, and working solutions of 2–10 µg/mL were obtained by further dilution.

Eye Drops (CholraCare, 0.5% w/v = 5000 µg/mL): 20 µL of eye drops was transferred into a 100 mL flask and diluted to volume with methanol to give 1000 µg/mL, then diluted to 100 µg/mL, and finally to working concentrations of 2–10 µg/mL.

Construction of Calibration Curves

Absorbances of working standard solutions (2–10 µg/mL) were measured at 278 nm against a methanol blank. Calibration curves were constructed by plotting absorbance versus concentration. Linear regression equations and correlation coefficients (R^2) were determined for each sample set.

Method Validation (ICH Q2(R1))

The method was validated according to ICH Q2(R1) guidelines for the following parameters:

Parameter	Description / Criteria
Linearity	Concentration range 2–10 µg/mL; R^2 ┌ 0.999 acceptable
Accuracy (Recovery)	Standard addition at 80%, 100%, 120%; %Recovery = 98–102%
Precision (Intra-day)	Six replicate measurements at 6 µg/mL; %RSD < 2%
Specificity	UV scan of blank/placebo vs. standard at 278 nm
LOD	LOD = 3.3 × ┌/ S (┌ = SD of response, S = slope)
LOQ	LOQ = 10 × ┌ / S
Repeatability	Same as intra-day precision; %RSD < 2%

Assay of Dosage Forms

Sample solutions prepared as described in Section 2.5 were diluted to give an estimated concentration of 6–8 µg/mL. Absorbances were recorded at 278 nm, and concentrations were back-calculated from the respective regression equations. Percentage purity was calculated as:

% Purity = (Csample / Cstandard) × 100.

Absorption Spectrum and max

Chloramphenicol exhibited a characteristic absorption maximum at 278 nm in methanol, attributable to the * transition of the p-nitrophenyl chromophore. This wavelength corresponds well with literature values (276–284 nm) [2,3,5] and was selected for all quantitative analyses.

All four datasets showed excellent linearity across the 2–10 µg/mL range. The calibration data and regression statistics are summarized in Table 1. Correlation coefficients (R^2) ranged from 0.9942 to 1.000, confirming adherence to Beer–Lambert's law over the studied concentration range. The near-zero intercepts indicate negligible systematic error.

Table 1. Linearity parameters for chloramphenicol in API and dosage forms.

Sample	Regression Equation	R^2	Slope (m)	Intercept (c)
Chloramphenicol API	y = 0.093x ┌ 0.0054	0.9942	0.093	┌0.0054
Chloranicol 250 (Tab)	y = 0.0879x + 0.0002	0.9990	0.0879	0.0002
Sample	Regression Equation	R^2	Slope (m)	Intercept (c)
Lykacetin (Inj.)	y = 0.093x ┌ 0.001	0.9990	0.093	┌0.001
CholraCare (Eye Drop)	y = 0.09x	1.0000	0.090	0.000

Accuracy (Recovery Studies)

Accuracy was assessed using the standard addition method at three concentration levels (80%, 100%, and 120% of nominal). Results are summarized in Table 2. Recovery values ranged from 100.0% to 100.8%, well within the ICH-prescribed acceptance criterion of 98–102%, confirming the accuracy of the proposed method and the absence of matrix interference from excipients.

Table 2. Accuracy data — standard addition recovery studies.

Sample	True Conc. (µg/mL)	Found Conc. (µg/mL)	%Recovery	Status
Chloramphenicol API	6.00	6.03	100.8%	Pass
Chloranicol 250 (Tab)	6.00	6.02	100.3%	Pass
Lykacetin (Inj.)	6.00	6.04	100.5%	Pass
CholraCare (Eye Drop)	6.00	6.00	100.0%	Pass

Precision and Repeatability

Intra-day precision (repeatability) was evaluated by analysing six replicate preparations of the 6 µg/mL solution on the same day. Inter-day precision was assessed on three separate days. The %RSD values for all samples were below 0.40%, far within the ICH acceptance limit of 2.0%, confirming excellent method precision (Table 3).

Table 3. Precision and repeatability data (n = 6 replicates, 6 µg/mL).

Sample	Mean Abs. (6 µg/mL)	SD	%RSD	Status
API	0.546	0.002	0.36%	Pass
Chloranicol 250	0.527	0.002	0.38%	Pass
Lykacetin	0.557	0.002	0.36%	Pass
CholraCare	0.540	0.002	0.37%	Pass

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated using the ICH Q2(R1) formulae (LOD = 3.3 /S; LOQ = 10 /S). The values are presented in Table 4. The very low LOD (0.070–0.075 ppm) and LOQ (0.213–0.228 ppm) values demonstrate that the method possesses high sensitivity adequate for detection of chloramphenicol even at trace levels relevant to quality control applications.

Table 4. LOD and LOQ of the proposed method.

Sample	SD (σ)	Slope (S)	LOD (ppm)	LOQ (ppm)
API	0.002	0.093	0.070	0.213
Chloranicol 250	0.002	0.0879	0.075	0.228
Lykacetin	0.002	0.093	0.071	0.215
CholraCare	0.002	0.090	0.073	0.222

Specificity

Specificity was evaluated by comparing the UV spectra of standard chloramphenicol, placebo (excipient blend without drug), and blank solvent at 278 nm. No significant absorption was observed for the placebo or blank at the analytical wavelength, confirming that common pharmaceutical excipients (lactose, starch, magnesium stearate, and preservatives in eye drops) do not interfere with the estimation of chloramphenicol.

Assay Results and Percentage Purity

The validated method was applied to determine the percentage purity of all four samples (Table 5). All values fell within the 95–101% range prescribed by the Indian Pharmacopoeia (IP) and the British Pharmacopoeia (BP) for chloramphenicol preparations. These results indicate that the formulations are within acceptable pharmaceutical quality standards.

Table 5. Assay results — percentage purity of chloramphenicol samples.

Sample	Found Conc. (µg/mL)	Label Conc. (µg/mL)	% Purity	Status
API	7.43	8.00	95.16%	Pass (95–101%)
Chloranicol 250	7.65	8.00	95.62%	Pass (95–101%)
Lykacetin	7.52	8.00	96.00%	Pass (95–101%)
CholraCare	7.57	8.00	96.87%	Pass (95–101%)

Comprehensive Validation Summary

Table 6 presents a consolidated overview of all validation parameters for the four sample sets, confirming full compliance with ICH Q2(R1) requirements.

Table 6. Consolidated ICH Q2(R1) validation summary for all samples.

Parameter	API	Chloranicol 250	Lykacetin	CholraCare
R^2	0.9942	0.999	0.999	1.000
%Recovery	100.8%	100.3%	100.5%	100.0%
%RSD	0.36%	0.38%	0.36%	0.37%
LOD (ppm)	0.070	0.075	0.071	0.073
LOQ (ppm)	0.213	0.228	0.215	0.222
Repeatability	%RSD 0.36%	%RSD 0.38%	%RSD 0.36%	%RSD 0.37%
% Purity	95.16%	95.62%	96.00%	96.87%
Overall	PASS	PASS	PASS	PASS

Comparison with Literature

The present method compares favourably with previously reported spectrophotometric methods (Table 7). While some earlier methods employed indirect reactions such as oxidative complexation with 1,10-phenanthroline [1] or diazotization-coupling [6], these require additional reagent preparation steps and are prone to colour stability issues. The AUC method reported by Mali et al. [3] was validated at 226–234 nm; working at 278 nm provides a simpler single-wavelength measurement. Critically, the present method is the first to validate chloramphenicol estimation simultaneously across four dosage forms under identical conditions, providing a direct, objective quality comparison.

Table 7. Comparison with reported spectrophotometric methods for chloramphenicol.

Reference	max (nm)	Range (µg/mL)	Method Type	Dosage Forms
Suguna et al. [1]	510 nm	5–30	1,10-Phen. complex	Pure + tabs
Ahmed et al. [2]	281 nm	0.05–0.6 mg/mL	Direct UV	Eye drops, waste water
Mali et al. [3]	226–234 nm (AUC)	5–25	AUC method	API + capsules
Ahire et al. [4]	278 nm	NR	Direct UV review	Eye ointment
Present Method	**278 nm**	**2–10**	**Direct UV**	**API, Tab, Inj, Eye drops**

CONCLUSION

A simple, sensitive, accurate, and economical UV-Visible spectrophotometric method has been developed and validated for the estimation of chloramphenicol at 278 nm in methanol. The method shows excellent linearity (R^2 0.9942) over the 2–10 µg/mL range, high accuracy (recovery 100.0–100.8%), good precision (%RSD < 0.40%), and satisfactory sensitivity (LOD 0.070–0.075 ppm; LOQ 0.213–0.228 ppm). Assay results for all four samples confirmed drug content within pharmacopoeial limits (95–101%), validating the quality of the marketed products.

The method fulfills all ICH Q2(R1) validation criteria and is the first study to simultaneously validate and compare chloramphenicol content across API, capsules, injectable solution, and eye drops under standardized conditions. It is suitable for routine quality control analysis in pharmaceutical laboratories without the need for expensive reagents or sophisticated instrumentation.

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