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Review Article

Analytical Method Development And Validation Of Chloramphenicol Eye Ointment By UV-Visible Spectroscopy: Review

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ABSTRACT

Chloramphenicol is used as eye drops or ointment to treat bacterial conjunctivitis. Chloramphenicol is effective against gram-positive and gram-negative bacteria, including many anaerobic microorganisms. It is widely used because it is cheap and easy. Two simple, rapid and sensitive spectrophotometric methods were developed for the determination of chloramphenicol (CAP) in pure and pharmaceutical form. Several methods (specificity, selectivity, linearity, sensitivity, precision, accuracy, and robustness) were used to analyze the presence of impurities, intermediates, and degradation products of chloramphenicol. The developed method is simple and easy to analyze explicit expressions. The proposed method has been successfully used to measure CAP in ointments and eye drops.

INTRODUCTION

Chloramphenicol is a bacteriostatic antibacterial compound first isolated by David Gottlieb and introduced into clinical practice in 1949 from Venezuelan Streptomyces bacteria. Its structure is shown in Figure 1. This is the first antibiotic produced synthetically on a large scale. Chloramphenicol is effective against various microorganisms. Chloramphenicol is used as eye drops or ointment to treat bacterial conjunctivitis [1]. Chloramphenicol is effective against Gram-positive and Gram-negative bacteria, including anaerobic microorganisms. It is widely used because of its cheapness and simplicity [2]. The most serious side effect associated with chloramphenicol therapy is bone marrow toxicity, which can occur in two forms. Immediate drug toxicity and bone marrow suppression are usually reversible, and aplastic anemia is unpredictable and dose-independent and usually fatal. CAP is non-irritating and is used primarily to treat skin, ear and eye infections such as trachoma [3].

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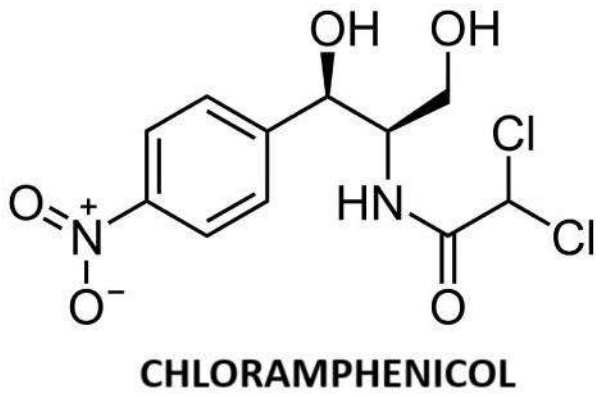

Figure 1: Chemical structure of Chloramphenicol.

Several methods have been used to determine the presence of chloramphenicol as impurities, intermediates, and degradation products. In 1976, Vigh and Inczédy reported an HPLC method to separate six chloramphenicol intermediates formed during preparation [4]. A method for the determination of chloramphenicol and monosuccinin in pig plasma was also described by Wiese et al. using RP-18 column with 30% methanol and 4.9% phosphate buffer as mobile phase [5]. Bohr and Peinenberg developed an HPLC method for the simultaneous determination of chloramphenicol and its most important degradation products. C18 reversed phase column using boric acid and acetonitrile phase change solution (60:40, v/v) at pH 3.0 [6]. Also, Seth and Banerjee identified chloramphenicol by the presence of degradation products of chloramphenicol in the formulation by polarographic method [7]. In 1991, Sudana and Chugar isolated chloramphenicol and benzocaine from impurities and compounds in topical solutions and extracts using the RP-HPLC method [8]. In 2001, an HPLC method for the analysis of gamma-irradiated chloramphenicol and impure chloramphenicol in ophthalmic ointment was described [9]. Chloramphenicol and glucuronides were found in several foods (livestock, seafood, and honey) by Bogus et al 2004. In 2006, Wiski et al. described an HPLC method to separate seven different drugs from their respective feces, namely chloramphenicol and faces [11]. To reliably measure the effect of chloramphenicol on gamma oxidation in ophthalmic ointment, chloramphenicol and degradation products must be accurately removed from the ointment base. Liquid-liquid extraction, solid-phase extraction, and centrifugation are commonly used to remove chloramphenicol from matrices. Compared with the conventional isolation method, chloramphenicol ophthalmic ointment, United States Pharmacopoeia (USP) and British Pharmacopoeia (BP) methods use liquid-liquid extraction with methanol and water as extractants [12]. Attia et al. presented an extraction method that considers the effect of ointment base and temperature on the stability of chlortetracycline hydrochloride and chloramphenicol in ophthalmic ointments. Kim and others, solid phase carbon black graphite was used to extract chloramphenicol from biological samples [13,14].

Chloramphenicol eye ointments:

Chloramphenicol is an antibiotic used topically to treat bacterial conjunctivitis. Bacterial conjunctivitis is caused by bacteria entering or entering the eye. This can cause burns, suffocation and bleeding. You will notice that your eyes produce irritating, watery mucus that sticks to your eyelashes [15]. Conjunctivitis is a common disease, but in some cases it can be cured without medication. However, for some people, the use of chloramphenicol is very effective and can significantly improve the condition of the eyes in a few days. Chloramphenicol is an antibiotic and therefore not suitable for treating non-bacterial eye infections such as viral eye infections [16,17].

Method development and validation of chloramphenicol by UV spectroscopy

UV-Vis spectroscopy is an analytical technique that measures the amount of different wavelengths of UV or visible light absorbed or transmitted by a sample compared to a reference or blank sample.

These properties affect the composition of the sample and provide information about what is present in the sample and in what concentration. Since this spectroscopy method depends on the use of light, let's first look at the properties of light [18] Light has a quantity proportional to its wavelength. Therefore, short wavelengths of light have more energy and long wavelengths have less energy. The amount of energy required to remove an electron from a substance to a higher energy state [19]. Different bonding environments in a material require different specific energies for electrons to move to higher energy states. Therefore, different materials absorb light at different wavelengths. Humans can see the visible light spectrum from 380 nm, which appears blue-green, to 780 nm, which appears red. Therefore, light can be described by wavelength, and finding the wavelength that corresponds to the maximum absorption in UV spectroscopy can help analyze or identify different substances (see applications in the UV spectroscopy section) [20,21].

General procedure for calibration:

An ideal method for the analysis of chloramphenicol residues should be sensitive, accurate and specific, and should provide uniform information on the identity of the analyte. In addition, it should be as cost-effective and reliable as possible. In practice, it is difficult to create a method that combines all these properties. Therefore, the analytical strategy used to monitor chloramphenicol residues in animal tissues often involves the first application of the test method followed by confirmatory analysis of samples that test positive for the test method [22].

Method A:

A volume (3-0.13 mL) of 100 µg/mL (3.1 x 10-4 M) chloramphenicol diazot is transferred to a series of 25 mL standard flasks. 4 ml of chromatotropic acid (3.1 x 10-4 M) and 2 ml of 4 M ammonia were added and mixed well. The contents of the vial were mixed with distilled water, mixed well, allowed to stand at room temperature for 15 minutes, and the red-violet absorbance was measured at 515 nm against the reagent containing all the contents except chloramphenicol. A calibration plot is generated and the regression equation is calculated to optimize the situation. All subsequent experiments used 2 mL diazotized 100 µg/mL chloramphenicol in a final volume of 25 mL [23].

Method B:

100µg.ml-1 (3.1 x 10-4M) diazotized chloramphenicol was transferred to a series of standard 25 ml bottles (0.1 - 4.5 ml). 1 ml of phenol (0.1%) and 1.5 ml of ammonia were added and mixed well. The content of the flask was filled with distilled water, mixed well and left at room temperature (10-15 ° C) for 15 minutes and the yellow absorbance was measured at 432 nm in the empty reagent containing all the components. Does not contain chloramphenicol. . . A calibration plot is generated and the regression equation is calculated to optimize the situation. All subsequent experiments used 2 mL of diazotized 100 µg/mL chloramphenicol in a final volume of 25 mL [23].

Solutions of pharmaceutical preparations:

Eye drops samples:

Mix the contents of the dropper bottle together. An aliquot (10 ml) corresponding to 50 mg of chloramphenicol was diluted to 50 ml with ethanol in a volumetric flask. Transfer this solution to a 125 ml beaker, reduce the volume as described above, and mix with 100 ml of distilled water to obtain 500 µg/mL-1 reduced chloramphenicol solution [23, 24].

Ointment samples:

Contains 5 tubes of ointment mixture and carefully measure the amount of ointment equal to 50 mg of chloramphenicol extract in 10 ml of ethanol. The solution was filtered and mixed with ethanol in a 50 ml volumetric flask. Transfer this solution to a 125 ml plate, reduce the volume as described

above and dilute to 100 ml of distilled water to obtain a 500 µg/ml-1 reduced chloramphenicol solution [23, 24].

Table 1: Analytical parameters for UV spectroscopy of chloramphenicol

Parameters	Method A	Method B
ʎ max (nm)	515	432
Linearity range (µg ml-1)	0.52-12.0	0.4-18.0
Molar absorptivity (L mol-1 cm-1)	1.241 × 104	1.491 × 104
Sandell's sensitivity (µg .cm-2)	26.03 × 10-3	21.67 × 10-3
Limit of detection (µg ml-1)	0.1334	0.0873
Relative standard deviation (RSD %)*	1.905	1.276
Stability (hr.)	2	2
Molar ratio (D:R)	1:1	1:1

VALIDATION METHOD:

Specificity and Selectivity:

Characterization is the process of evaluating analytes for the presence of components expected to be present in the sample matrix. Sampling is the process of qualitatively determining the presence of analytes in the presence of elements that are expected to be present in the sample matrix. Pre-weighed drugs are added to the formulation, absorption is measured, and calculations are made to determine the amount of drug [25, 26].

Linearity:

To evaluate the linearity of the method, the standard mixture solution prepared as described in 2.4 was injected into the capillary and analyzed. The linearity between the concentration and the peak level of each analyte was assessed by the least square linear regression method, and if $P < 0.05$, the significance of the linear regression was confirmed by a one-way analysis of variance test [27].

Sensitivity:

According to the current ICH Q2R1 guidelines [28], limit of detection (LOD) and limit of quantification (LOQ) are not required for quantitative methods. However, the method must ensure that the working concentration for each analyte is below the LOQ. Therefore, the limit of quantification (LOQ) for each analyte was determined by analyzing solutions containing different concentrations of chloramphenicol, methylparaben, and propylparaben and measuring the signal-to-noise ratio for each analyte. The limit of quantification (LOQ) is the concentration with a signal-to-noise ratio of 10:1 and an RSD of triplicate analysis of less than 10% [29].

Accuracy:

The recovery test was performed by applying unlabeled chloramphenicol (standard dose method) to a sample of the formulation containing the drug. The recovery test is performed using a biological sample (blood) containing a specific amount of chloramphenicol corresponding to 2 mg of the patient's preparation. 2 mg is added using the standard addition method indicated. After adding this standard, transfer the contents to a 100 ml volumetric flask and let it dissolve in the solution. I finally signed up to be a speaker. "Whatman No. 41" solution was filtered through filter paper. The mixed sample solution was analyzed and the absorbance was measured. Five decisions are made in each recovery and current period [25,30].

Precision:

The accuracy of the CZE method was tested in terms of system consistency, repeatability, and interval accuracy [31,32]. System suitability was determined by measuring mixed standard solutions containing each analyte at 100% concentration six times a day [15]. Repeatability

and precision intervals were determined using six measurements in one day and two separate days at approximately 100% working concentration containing an analyte [33,34].

Repeatability:

A blank standard solution of chloramphenicol was prepared to measure the absorbance against the solution. Adhesion to solutions of the same concentration was measured five times and the standard deviation was calculated and expressed [25].

Interferences studies:

Inert, the formulation contains several ingredients to test chloramphenicol under optimal conditions. Although the formulation contains more layers than expected, none of them interfere with the proposed mechanism [25,31].

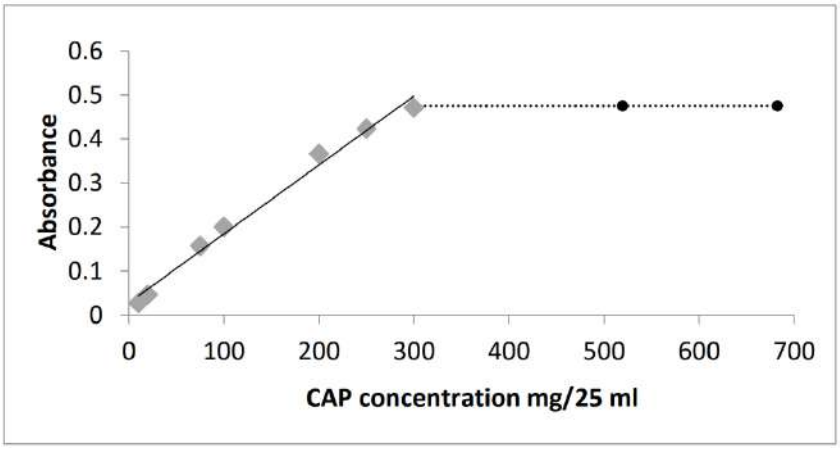

Figure 2: Calibration graph for the determination of chloramphenicol using the proposed method

Pharmaceutical application:

The proposed method was successfully compared with pure chloramphenicol and the British Pharmacopoeia for the drug [23]. The results of the proposed method for the tested formulations are in accordance with the published literature. The results in Table 2 were obtained by a formal method [23] with a confidence level of 95% using F test and t-test. For methods A and B, the calculated values of F and t do not exceed the critical values of F and t, respectively. This confirms that there is no significant difference between the proposed method and the official method in terms of accuracy and precision in determining chloramphenicol in drugs [23].

CONCLUSION:

The proposed method (UV Spectroscopy) is simpler, faster, cheaper and more selective than some reported methods. It is accurate and has the advantage of not requiring sample removal, chemical sample preparation, temperature control, pH control, extraction steps, or expensive reagents or solvents. The proposed method can be used to analyze chloramphenicol in pharmaceutical formulations and routine analysis. The proposed method for pure and pharmaceutical chloramphenicol was successfully compared with the British Pharmacopoeia. For all preparations studied, the results of the proposed method are in agreement with published materials.

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