

An Alternative Method for Determination of Chloramphenicol in Pharmaceutical Formulation by Laser Fluorimeter Analyser

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Abstract: In this article, Chloramphenicol (CHP) was determined via local made spectrofluorometric using solid state laser diode 405 nm as a source combined with flow injection technique. The proposed method is characterized by sensitive, accurate and speed for the determination of CHP in different pharmaceutical formulations. The procedure depends on the fluorescence of 2,7-dichlorofluorescein as a fluorescent dye and quenching fluorescence effect by chloramphenicol in continuous system. All the optimum parameters like fluorescent dye concentration, pH, flow rate, sample volume, and coil addition have been investigated. The CHP dynamic range was 0.1-3.0 mMol.L⁻¹ with correlation coefficient (r) =0.9836 while the (%r²) percentage linearity was %96.75. Limit of Detection is depended on the dilution of minimum concentration in calibration curve 1.680 µg/Sample. The repeatability for 1 and 3 mMol.L⁻¹ of CHP solution was found to be less than 1% (n=6). The proposed method was successfully applied to determine the CHP in pharmaceutical formulations (capsules and eye drop).

Keywords: chloramphenicol, spectrofluorometric, 2,7-dichlorofluorescein, laser diode 405 nm, flow injection analysis

1. Introduction

Chloramphenicol (CHP) is chemically named 2,2 dichloro-N-[(1R,2R)-2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl) ethyl] acetamide, with molecular structure C₁₁H₁₂C₁₂N₂O₅, and the chemical structure shown in Figure (1). Its molecular weight 323.126 g.mol⁻¹, it is a white, grayish-white or yellowish-white, fine crystalline powder or fine crystals, freely soluble in methanol, ethanol, butanol, ethyl acetate, acetone, and in propylene glycol, slightly soluble in water⁽¹⁾.

Chloramphenicol is considered a prototypical broad-spectrum antibiotic. It is effective against a wide variety of and Gram-negative Gram-positive bacteria, including most anaerobic organisms⁽²⁾.

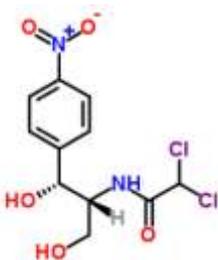


Figure 1: Chemical structure of Chloramphenicol

The literature survey detects various of analytical methods which have reported for determination of Chloramphenicol using UV-Visible Spectrophotometric⁽³⁻⁶⁾, FIA-Spectrophotometric⁽⁷⁾, FIA-chemiluminescence⁽⁸⁻¹¹⁾, High Performance Liquid Chromatography⁽¹²⁻¹³⁾, Resonance Rayleigh scattering⁽¹⁴⁾ and spectrofluorometric method⁽¹⁵⁾.

This paper describes a sensitive, simple and accurate fluorimetric method combined with flow injection technique for analysis of Chloramphenicol in pharmaceutical preparations. The proposed method uses 2,7-dichlorofluorescein as a fluorescent dye which forms

constant fluorescence intensity then fluorescence is quenched by chloramphenicol in aqueous medium⁽¹⁶⁻¹⁹⁾. The fluorescence and quenching are measured via local made laser diode fluorimeter combined with continuous flow injection.

2. Materials and Methods

Chemicals

All chemicals that used in this were of analytical grade and distilled water was used in all dilution processes. A standard solution of sodium carbonate (BDH) (Na₂CO₃, 105.99 g.mol⁻¹) (0.01 mol.L⁻¹) was prepared by dissolving 1.0599g in 1000 mL distilled water. Stock solution of sodium hydroxide (BDH) (NaOH, 40 g.mol⁻¹) (0.1 mol.L⁻¹) was prepared by dissolving 1.00 g of the base in distilled water and dilute to the mark with the same solvent to 250 mL calibrated flask.

Pure grade 2,7-dichlorofluorescein was purchase from Riedel De Haen AG Seelze Hannover, standard stock solution of 2,7-dichlorofluorescein (C₂₀H₁₀Cl₂O₅, 401.21 g.mol⁻¹) (0.001 mol.L⁻¹) was prepared dissolving 0.4012 g in 0.01M Na₂CO₃ and complete to mark with same base.

Stock solution of Chloramphenicol (Sigma-Aldrich) (C₁₁H₁₂C₁₂N₂O₅, 323.12 g.mol⁻¹) (0.01 mol.L⁻¹) was prepared by dissolving 0.3231 g in 30 mL of 0.1 M sodium hydroxide then completed to 100 mL with distilled water, the dark container was used to keep solution and kept in refrigerator.

3. Apparatus

Local made Laser diode fluorimeter instrument was used to measure fluorescence with excitation via laser diode at 405nm. Photo diode was used as the detector with a vertical position at 90 angle with radiation source.

Volume 6 Issue 8, August 2017

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Single channel variable speed Ismatec (ISM796) peristaltic pump was used to fluid propulsion, a Rheodyne injection valve (2-directions, 6-port) with a sample loop (0.5 mm i.d., Teflon, variable length) used for sample injection.

Siemens x-t potentiometric recorder (KOMPENSO GRAPH C-1032) and Digital AVO-meter were used to record the output signals. Peak height was measured for each signal. UV-Vis spectra were measured on SHIMADZU spectrophotometer model 1800 (Japan). Figure (2) shows the flow system that used for the determination and detection of chlorpromazine HCl.

4. Methodology

The whole manifold system Figure (2) used for determination of chloramphenicol in different pharmaceutical formulations. The procedure involves reaction of chloramphenicol with 2,7-dichlorofluorescein to form an fluorescence quenching of reference fluorescent material.

The manifold reaction design is composed of the carrier stream (2,7-dichlorofluorescein 0.1 mMol.L^{-1}) that gives a constant and continuous emission of fluorescence light at 1.6 mL/min flow rate which lead to the injection valve to carry Chloramphenicol sample segment with sample volume $135 \text{ }\mu\text{L}$. The mixture was then passes through the measuring cell that gives quenching fluorescence response which was recorded on x-t potentiometric auto AVO-meter.

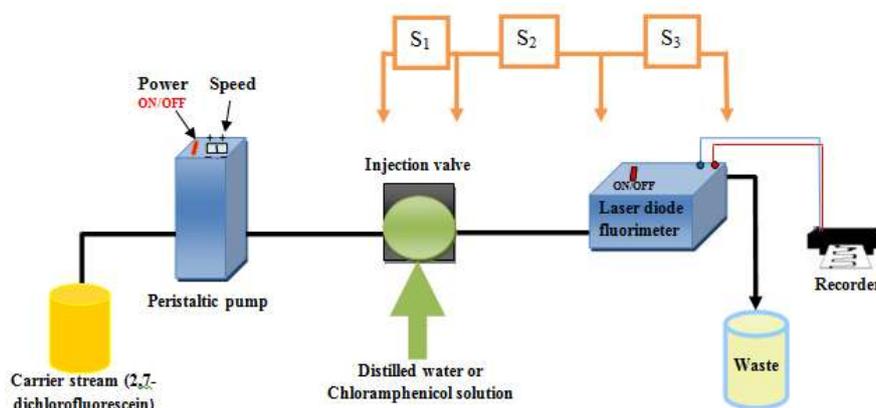


Figure 2: Flow gram of manifold used to determination of chloramphenicol based on fluorescence quenching

4.1 Variable Optimization

The chemicals and physical parameters such as 2,7-dichlorofluorescein concentration, phosphate buffer effect, flow rate, sample volume, delay coil were investigated to establish the optimum parameters.

4.2 Chemical Parameters

1- Variation in 2,7-dichlorofluorescein Concentration

Single line manifold system was used at flow rate 1.6 mL.min^{-1} for carrier stream (2,7-dichlorofluorescein) at

concentration range ($0.01\text{-}0.5 \text{ mMol.L}^{-1}$) and inject the Chloramphenicol drug in the sample loop ($135 \text{ }\mu\text{L}$) at preliminary concentration 3 mMol.L^{-1} , after mixing, the mixture arrives to the flow cell which was irradiated by blue-violet laser diode fluorimeter. It was noted that an increase in the 2,7-dichlorofluorescein concentration leads to an increase in the continuous fluorescence intensity. Figure (3) shows the response profile of variation of 2,7-dichlorofluorescein and fluorescence quenching for both of water and drug. Therefore; 0.3 mMol.L^{-1} 2,7-dichlorofluorescein concentration was chosen as the optimum concentration that applied for after time tests.

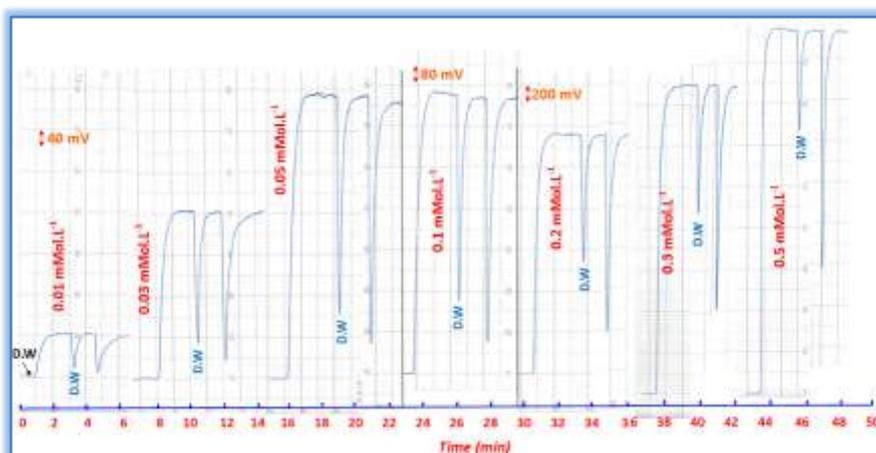


Figure (3): Response profile of variation for 2,7-dichlorofluorescein concentrations and fluorescence quenching of water and chloramphenicol

2-Variation of pH of Phosphate Buffer Solution

The effect of phosphate buffer solutions was examined using a series of solutions, the pH values were measured and found to be as follows 3.5, 4.5, 6, 7, 8 and 9 which were prepared and adjusted with phosphoric acid solution or with strong sodium hydroxide solution. The experiment was carried out using the optimum concentration (0.3 mMol.L⁻¹) of 2,7-dichlorofluorescein, and preliminary 3 mMol.L⁻¹ of Chloramphenicol with 135µL sample volume. It was noticed a slight decrease in fluorescence intensity at higher value (pH > 6) and low value (pH < 6) and decrease in drug quenching response. The quenching effect of pH on drug

response might be forming a drug salt that lead to form a little precipitate of drug when use different pH (acidic or alkaline).

The obtained results were tabulated in Table (1), which summarize the average of three successive readings with relative standard deviation and confidence interval of the average response at 95% confidence for fluorescence quenching. Therefore, it was ascertained that pH was not useful to give the best response in the fluorescence quenching and the most suitable at compromise for have the maximum fluorescence intensity was distilled water.

Table 1: Effect of pH on the measurements of fluorescence response

| pH | Continuous of fluorescence response (n=3) \bar{y}_i in mV | Total fluorescence quenching (n=3) \bar{y}_i in mV | σ_{n-1} | RSD% | Confidence interval of the average at (95%) $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$ | Fluorescence quenching by distilled water in mV | Fluorescence quenching of CHP \bar{y}_{O_i} (n=3) in mV | Fluorescence remaining \bar{y}_{R_i} (n=3) in mV |
|-----|---|--|----------------|------|--|---|---|--|
| D.W | 3740 | 2720 | 31.18 | 1.14 | 2720±77.454 | 1520 | 1200 | 1020 |
| 3.5 | 655 | 360 | 10.00 | 2.78 | 360±24.843 | 331 | 29 | 295 |
| 4.5 | 3809 | 1137 | 8.00 | 0.70 | 1137±19.875 | 872 | 265 | 2672 |
| 6 | 4000 | 1200 | 17.32 | 1.44 | 1200±43.030 | 980 | 220 | 2800 |
| 7 | 3791 | 1111 | 3.51 | 0.32 | 1111±8.725 | 906 | 205 | 2680 |
| 8 | 3751 | 1237 | 14.73 | 1.19 | 1237±36.597 | 1117 | 120 | 2514 |
| 9 | 3432 | 1020 | 10.00 | 0.98 | 1020±24.843 | 588 | 432 | 2412 |

4.3 Physical Parameter

1- Sample Volume

Using variable volume of sample segment (3 mMol.L⁻¹ Chloramphenicol) at injection valve extended from 46-206µL, were studied with open valves mode. The optimum concentration 0.3mMol.L⁻¹ of 2,7-dichlorofluorescein solution

at flow rate 1.6 mL/min for carrier stream. It was noticed that an increase in sample volume up to 65 µL lead to increasing of fluorescence quenching of Chloramphenicol response. Table (2) shows an increasing in the sample volume lead to a significant increase in sensitivity and gave regular fluorescent responses. Therefore; 65µL was chosen as an optimal sample volume.

Table 2: Variation of injected sample volume on the measurement of fluorescence response

| length of sample loop (cm) Diameter (0.5mm) | Injected sample loop volume (µl) | Total fluorescence quenching (n=3) \bar{y}_i in mV | σ_{n-1} | RSD% | Confidence interval of the average at (95%) $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$ | Fluorescence quenching by distilled water in mV | Fluorescence quenching of CHP \bar{y}_{O_i} (n=3) in mV | Fluorescence remaining \bar{y}_{R_i} (n=3) in mV | Base width Δt_B (sec.) |
|--|----------------------------------|--|----------------|------|--|---|---|--|--------------------------------|
| 12 | 46 | 2101 | 12.12 | 0.58 | 2301±28.651 | 940 | 1161 | 1639 | 30 |
| 17 | 65 | 2301 | 11.53 | 0.50 | 2720±43.030 | 1014 | 1287 | 1439 | 42 |
| 25 | 96 | 2600 | 20.00 | 0.77 | 2732±49.687 | 1360 | 1172 | 1008 | 48 |
| 35 | 135 | 2720 | 17.32 | 0.64 | 2739±21.515 | 1520 | 1200 | 1020 | 54 |
| 40.9 | 157 | 2739 | 11.53 | 0.42 | 2780±52.584 | 1600 | 1139 | 1001 | 66 |
| 53.6 | 206 | 2780 | 21.17 | 0.76 | 65.729±3340 | 1680 | 1100 | 960 | 74 |

2- Flow rate

A set of experiments were carried out for the optimization of flow rate of carrier stream (2,7-dichlorofluorescein solution) using 3 mMol.L⁻¹ Chloramphenicol, 65 µL as sample volume and at a variable flow rate. Figure (4) shows that at low flow rate there is an increase in peak height and base width for response expressed as total quenched fluorescence (includes response of Chloramphenicol with distilled water),

this might be attributed to the increase dispersion and dilution effect due to the distribution of segment. While the high flow rate leads to decrease in peak height, decrease the peak base width, decrease analysis time and minimize dilution effect. Therefore, the best flow rate was 1.3 mL/min which gave relatively suitable response and used throughout all applications.

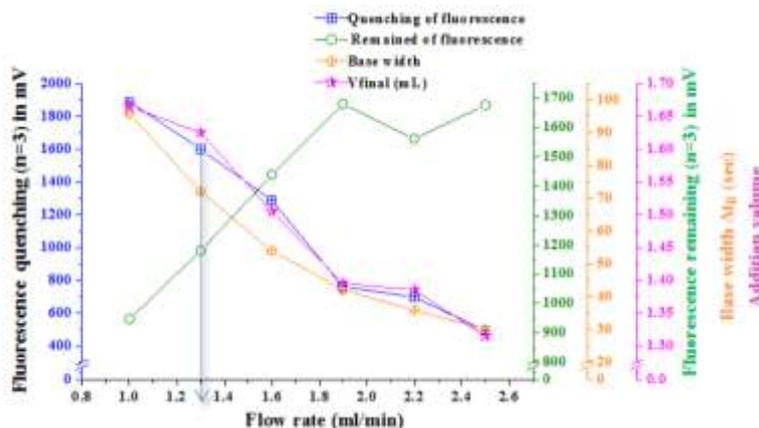


Figure 4: Effect of flow rate on Fluorescence quenching, Fluorescence remaining, peak base and addition volume

3- Reaction Coil

The coil was settled after injection valve position, to mix the solutions. The studied was performed using optimum concentration for 2,7-dichlorofluorescein solution 0.3 mMol.L^{-1} , preliminary concentration Chloramphenicol 3 mMol.L^{-1} and physical parameters achieved (sample loop $65 \mu\text{L}$ at flow rate 1.3 mL/min with open valve) in previous

studies with variable coil volume (0-206) μL . The obtained results are tabulated in Table (3). It was noticed there were a decrease in the fluorescence quenching of CHP during use reaction coil this might be attributed to the dilution and dispersion effect. A study was carried out and noticed no coil was used in manifold that gave a high and repeatable response profile.

Table 3: Variation of coil length on total fluorescence quenching response expressed as an average peak heights (n=3)

| Coil length (cm) | Coil volume (μl)= $r2\pi h$ | Total fluorescence quenching (n=3) \bar{y}_i in mV | σ_{n-1} | RSD% | Confidence interval of the average at (95%) $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$ | Fluorescence quenching by distilled water in mV | Fluorescence quenching of CHP \bar{y}_{Qi} (n=3) in mV | Fluorescence remaining \bar{y}_{Ri} (n=3) in mV |
|------------------|--|--|----------------|------|--|---|--|---|
| 0 | 0 | 2560 | 20.00 | 0.78 | 2560 ± 49.687 | 960 | 1600 | 1180 |
| 10 | 39 | 2372 | 17.447 | 0.74 | 2372 ± 43.316 | 961 | 1411 | 1368 |
| 15 | 58 | 2400 | 15.00 | 0.63 | 2400 ± 37.265 | 1100 | 1300 | 1340 |
| 25 | 96 | 1720 | 5.00 | 0.29 | 1720 ± 12.422 | 780 | 940 | 2020 |
| 35 | 135 | 1398 | 15.87 | 1.14 | 1398 ± 39.438 | 622 | 776 | 2342 |
| 40.9 | 157 | 1398 | 10.82 | 0.77 | 1398 ± 26.872 | 780 | 618 | 2342 |
| 53.6 | 206 | 1380 | 15.62 | 1.13 | ± 38.8071380 | 760 | 620 | 2360 |

5. Response of continuous fluorescence: 3740mV

Variation of Chloramphenicol Concentration

A series of Chloramphenicol solution ranging from $0.1-4.5 \text{ mMol.L}^{-1}$ were prepared in order to construct the calibration curve (Figure (5)). All physical and chemical variables were fixed at their optimum values. Table (4) illustrates the brief results of determination of Chloramphenicol using laser diode fluorimeter-flow injection and classical method spectrophotometers shows the values of correlation coefficient

the percentage of linearity ($\%r^2$), and the calculated t-values at confidence interval of 95% for the dynamic calibration curve. A straight line ranging from $(0.1-3) \text{ mMol.L}^{-1}$ with correlation coefficient (r): 0.9836 with proposed method. While the UV-Spectrophotometric (classical method) at $\lambda_{\text{max}}=278 \text{ nm}$, direct measurement (The method outlined in the British Pharmacopoeia⁽¹⁾ was adopted) the calibration curve was established to estimation of Chloramphenicol from $(0.0001-0.05) \text{ mMol.L}^{-1}$ with correlation coefficient (r): 0.9991.

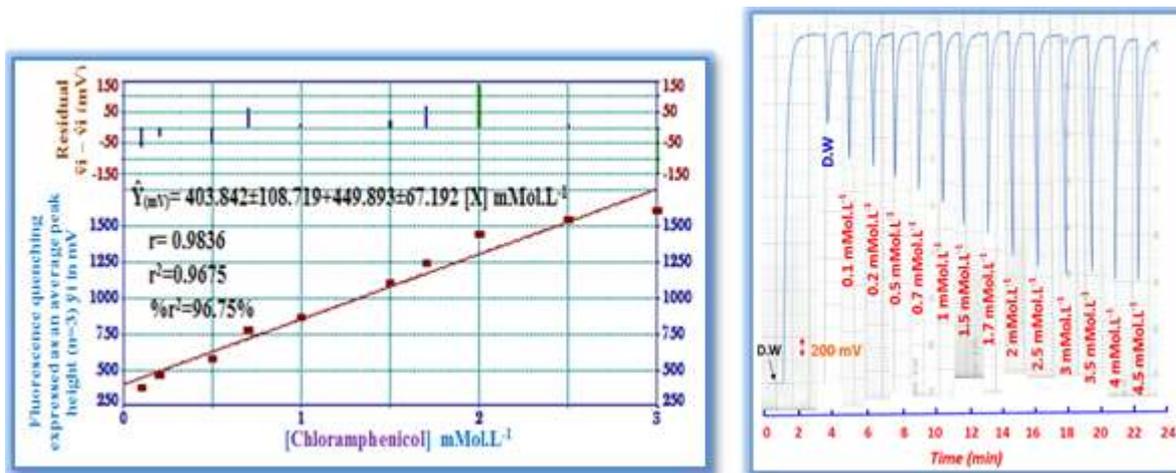


Figure 5: Calibration graph for the variation of Chloramphenicol concentration and response profile using laser diode fluorimeter-flow injection

Table 4: Summary of calibration graph results for the determination of CHP using developed method (laser diode fluorimeter) and classical method spectrophotometer

| Measured [Chl.] mMol.L ⁻¹ | Liner dynamic range mMol.L ⁻¹ | Type of measurement | $\hat{Y}_{(mv)} = (a \pm S_{a,t}) + (b \pm S_{b,t})$ [Chloramphenicol] mMol.L ⁻¹ at confidence level 95%, n-2 | r r ² %r ² | t _{tab} at 95% confidence level, n-2 | Calculated t-value $t_{cal} = \frac{r/\sqrt{n-2}}{\sqrt{1-r^2}}$ |
|--------------------------------------|--|-------------------------------|--|--|---|---|
| 0.1-4.5 | 0.1-3.0 n=10 | Total fluorescence quenching | $\hat{Y}_{(mv)} = 1377.851 \pm 46.036 + 439.247 \pm 160.065[X]$ mMol.L ⁻¹ | 0.9836 0.9675 %96.75 | 2.306 << 43.643 | |
| | | Fluorescence Quenching of CHP | $\hat{Y}_{(mv)} = 57.851 \pm 46.036 + 439.247 \pm 160.065[X]$ mMol.L ⁻¹ | | | |
| | | Fluorescence Remaining | $\hat{Y}_{(mv)} = 2362.149 \pm 46.036 - 439.247 \pm 160.065[X]$ mMol.L ⁻¹ | | | |
| 0.0001-0.05 | 0.0001-0.05 n=12 | Absorbance | $\hat{Y} = 0.006 \pm 0.005 + 9.959 \pm 0.300 [X]$ mMol.L ⁻¹ | 0.9991 0.9982 %99.82 | 2.228 << 235.637 | |

\hat{y} : estimated response (mV) for n=3 expressed as an average peak height of linear equation of the form $\hat{y} = a + bx$ or absorbance, r : correlation coefficient, r²: coefficient of determination, r² %: linearity percentage.

Analysis of variance was carried out as shown in Table (5) which indicated that $F_{tab} \ll F_{Stat} = 238.3972$ therefore, it can be concluded that there is a significant relation between the concentrations of chloramphenicol and the response obtained.

Table 5: ANOVA for regression equation results⁽²⁰⁻²¹⁾

| Source | Sum of squares | D _f | Mean square | $F_{stat} = S_1^2 / S_2^2$ |
|---------------------------------|----------------|-------------------|-------------|----------------------------|
| Regr. ($\bar{y}_i - \bar{y}$) | 1772244 | V ₁ =1 | 1772244.018 | 238.3972 |
| Error ($y_i - \bar{y}_i$) | 59471.99 | V ₂ =8 | 7433.998145 | |
| Total ($y_i - \bar{y}$) | 1831716 | 9 | | |

Limit of Detection (L.O.D)

Table 6: Limit of detection for the determination of chloramphenicol at optimum parameter

| Minimum concentration (mMol.L ⁻¹) | Practically based on minimum concentration in calibration graph | Theoretical based on the value of slope L.O.D = 3S _B /slope | Based on the linear equation $\hat{Y} = Y_B + 3S_B$ |
|---|---|---|---|
| 0.08 mMol.L ⁻¹ | 1.680 µg/Sample | 0.035 µg/Sample | 12.076 µg/Sample |

Repeatability

The repeatability and efficiency of laser diode fluorimeter with continuous flow injecting were studied at 1

Limit of detection for Chloramphenicol was carried out by three different methods at sample volume 65 µL. The results were tabulated in Table (6).

1. Gradual dilution:

Based on successive dilution of the lowest concentration that used in calibration graph, this should be regarded as the real.

2. Theoretically (slope method):

L.O.D. = 3S_B / slope

S_B = σ_{(n-1)B} (standard deviation of blank n=13)

3. Theoretically (Linear equation) method:

$\hat{Y} = Y_B + 3S_B$
 \hat{Y} = Estimated value, Y_B (average response for the blank solution, this is equivalent to intercept (a) in straight line equation $y = a + bx$).

and 3 mMol.L⁻¹ of Chloramphenicol. The measurements were carried out at optimum parameters for six successive injections. The all results are tabulated in Table (7). Figure

(6) show the response profile of repeatability at 1 and 3 mMol.L⁻¹ respectively. The percentage relative standard

deviation is less than 1% exhibit the trustiness of the newly methodology.

Table 7: Repeatability of the determination of CHP using homemade laser diode Fluorimeter

| [CHP] mMol.L ⁻¹ | Total fluorescence quenching (n=6) \bar{y}_i in mV | Fluorescence quenching of CHP \bar{y}_{0i} (n=6) in mV | σ_{n-1} | RSD% | Confidence interval of the average at (95%) $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$ | Number of injection |
|----------------------------|--|--|----------------|------|--|---------------------|
| 1 | 1820 | 500.417 | 1.00 | 0.20 | 1.053±1820 | 6 |
| 3 | 2560 | 1239.833 | 0.82 | 0.07 | 2560±0.857 | 6 |

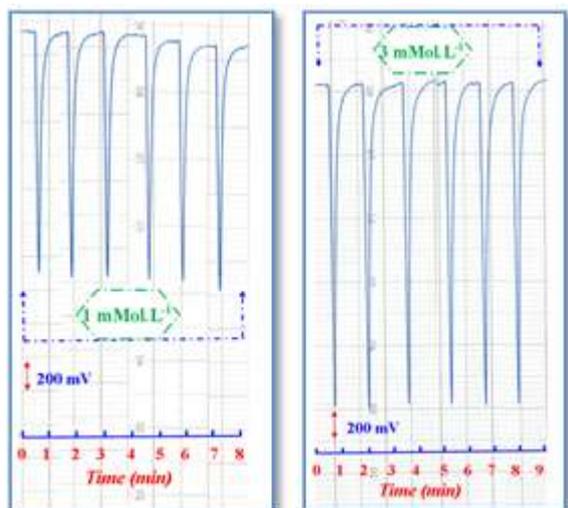


Figure 6: A profile of six successive measurements of 1 and 3 mMol.L⁻¹ of Chloramphenicol

The Applications

The adopted method achieved in this work was used for the determination of Chloramphenicol in different of pharmaceutical formula (capsule and eye drops). The capsules and eye drops were commercially purchased. Twenty capsules were weight and collective in container

then a weighing an amount equivalent 0.1850 g (equivalent to 0.1616 g of active ingredient) for Chloramphenicol-India. Distilled water used to dissolve the weighing powder to prepare 0.005 mol.L⁻¹. While the eye drops were purchased from four different companies. The volume of drops were measured and collected in dark container. 0.4 mL was drawing to prepare 0.005 mol.L⁻¹ and then dilution with distilled water. The development method was compared with official spectrophotometric method through the measurement of λ_{max} at 278 nm. The standard addition method was applied by preparing a series of solutions via transferring 0.4 mL (5 mMol.L⁻¹) to five volumetric flasks (10 mL) followed by the addition of gradual volumes of standard Chloramphenicol solution (5 mMol.L⁻¹) (0, 1, 1.4, 2 and 3 mL) in order to have the concentration (0, 0.5, 0.7, 1 and 1.5 mMol.L⁻¹) to constructed the standard addition curve. The results were tabulated in Table (8-A). While paired t-test of newly developed method with classical method shown in Table (8-B). The obtained results show the calculated t-value which is less than tabulated t-value which indicated no significant difference between the fluorescent method and the classical method.

Table (8-A): Summary of results by standard additions method for the determination of Chloramphenicol by Laser Diode Fluorimeter and Absorbance methods.

| Sample No. | Commercial name, Country, Content, Company, Drug form | Confidence interval for the average tablet weight $\bar{W} \pm 1.96 \frac{\sigma_{n-1}}{\sqrt{n}}$ (g) | Sample weight equivalent to 0.1616 g (5 mMol.L ⁻¹) of the active ingredient (g) | Theoretical content of active ingredient at 95% (g) | Development method using Laser diode Fluorimeter | | |
|------------|---|--|---|---|--|---|---|
| | | | | | Equation of standard addition curve at 95% for n-2 $\hat{Y}_{(mV)} = (a \pm S_{a,t}) + (b \pm S_{b,t})$ [Chloramphenicol] mMol.L ⁻¹ | r r ² r ² % | Practical concentration mMol.L ⁻¹ in 10 ml, 100 mL |
| 1 | Chloramphenicol, India, 250 mg, BRAUN Capsule | 0.286±0.0029 | 0.185 | 0.25±0.0026 | $\hat{Y}_{(mV)} = 209.139 \pm 239.904 + 930.623 \pm 268.558 [X] \text{ mMol.L}^{-1}$ | 0.9759 0.9879 98.79% | 0.225 5.618 |
| | | | | | $\hat{Y} = 0.016 \pm 0.013 + 10.750 \pm 2.673 [X] \text{ mMol.L}^{-1}$ | 0.9820 0.9909 99.09% | 0.015 7.256 |
| Sample No. | Commercial name, Country, Content, Company, Drug form | Theoretical Content wt./v (g/100ml) Concentration M | Prepared Conc. (mMol.L ⁻¹) | | Equation of standard addition curve at 95% for n-2 $\hat{Y}_{(mV)} = (a \pm S_{a,t}) + (b \pm S_{b,t})$ [Chloramphenicol] mMol.L ⁻¹ | r r ² r ² % | Practical concentration mMol.L ⁻¹ in 10 ml, 100 mL |
| | | | After drawing 32.468 ml | After drawing 0.4 ml | | | |
| 2 | Chloramphenicol, Jordan, 0.5%, API, Eye drop | 0.5% 0.015 M | 5 mMol.L ⁻¹ | 0.2 mMol.L ⁻¹ | $\hat{Y}_{(mV)} = 190.147 \pm 126.965 + 871.693 \pm 142.130 [X] \text{ mMol.L}^{-1}$ | 0.9922 0.9961 | 0.218 5.453 |
| | | | | | $\hat{Y} = 0.013 \pm 0.005 + 10.150 \pm 0.914 [X] \text{ mMol.L}^{-1}$ | 0.9976 0.9988 | 0.0013 6.502 |

| | | | | | 99.88% | |
|---|--|-----------------|--|--|----------------------------|-----------------|
| 3 | Chloramphenicol, Iraq, 0.5%, SDI, Eye drop | 0.5% 0.015 M | 5 mMol.L ⁻¹ 0.2 mMol.L ⁻¹ | $\hat{Y}_{(mV)}=189.583\pm 241.682+918.131\pm 270.546 [X] \text{ mMol.L}^{-1}$ | 0.9749 0.9874 98.74% | 0.206 5.162 |
| | | | | $\hat{Y}=0.016\pm 0.024+10.900\pm 4.850 [X] \text{ mMol.L}^{-1}$ | 0.9446 0.9719 97.19% | 0.0015 7.248 |
| 4 | Chloramphenicol, Belgium, 0.5%, RAMEDA, Eye drop | 0.5% 0.015 M | 5 mMol.L ⁻¹ 0.2 mMol.L ⁻¹ | $\hat{Y}_{(mV)}=195.340\pm 181.823+913.594\pm 203.537 [X] \text{ mMol.L}^{-1}$ | 0.9855 0.9927 99.27% | 0.214 5.345 |
| | | | | $\hat{Y}=0.014\pm 0.005+10.200\pm 1.023 [X] \text{ mMol.L}^{-1}$ | 0.9970 0.9985 99.85% | 0.0014 6.961 |
| 5 | Chloramphenicol, China, 0.5%, KONTAM, Eye drop | 0.5% 0.015 M | 5 mMol.L ⁻¹ 0.2 mMol.L ⁻¹ | $\hat{Y}_{(mV)}=184.032\pm 272.338+944.281\pm 304.864 [X] \text{ mMol.L}^{-1}$ | 0.9700 0.9849 98.49% | 0.195 4.872 |
| | | | | $\hat{Y}=0.013\pm 0.012+9.550\pm 2.551 [X] \text{ mMol.L}^{-1}$ | 0.9793 0.9896 98.96% | 0.0014 6.911 |

\hat{y} : Estimated response value (mV for laser diode fluorimeter and pH-meter method) for (n=3), method, r :correlation coefficient, r²: coefficient of determination & r²%: linearity percentage, $t_{0.05/2, 2} = 4.303$. UV –Sp.: UV –spectrophotometric method, $t_{0.025, \infty} = 1.96$ at 95%

Table (8-B): Paired t-test results and efficiency of determination for laser diode fluorimeter method with quoted value for the determination of Chloramphenicol in pharmaceutical formulations

| Sample No. | Practical content (mg) | | Efficiency of determination (% Rec) | Paired t-test $t = \frac{\bar{X}_d - \bar{X}_n}{\sigma_{n-1}}$ | ttab. at 95% confidence interval n-1 |
|------------|------------------------|--------------------|-------------------------------------|--|--------------------------------------|
| | Quoted value | Development method | | | |
| 1 | 0.250 g | 0.281 g | 112.35 | 3.16 < 4.303 | |
| 2 | 0.5% | 0.54% | 108.00 | 2.32 < 4.303 | |
| 3 | 0.5% | 0.51% | 102.00 | 2.94 < 4.303 | |
| 4 | 0.5% | 0.53% | 106.00 | 2.56 < 4.303 | |
| 5 | 0.5% | 0.48% | 96.00 | 2.44 < 4.303 | |

6. Conclusion

The fluorescence measurement is conducted by Laser Diode fluorimeter-CFI analyser for determination of CHP. The developed method is characterized by sensitive, accurate and speed. The fluorescence method is based on the continuous creation of fluorescence for 2,7-dichloro fluoresce in at on constant level intensity, then the measurement of fluorescence quenching after injected of CHP can lead to reduction in fluorescence intensity. A good precision of the proposed method was observed for all samples via value of %RSD less than 1%. Matrix effects were canceled by standard addition method. Therefore, the fluorescence method can consider as an alternative method for determination of Chloramphenicol in pharmaceutical preparations.

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