Determination of Chloramphenicol from pure and pharmaceutical preparations using FIA-Spectrophotometric methods

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Abstract

Flow injection spectrophtometric method was described for the trace determination of chloramphenicol. This method depends upon the reduction of the nitro group of the chloramphenicol to the amino group using Zn- reductor mini-column, then diazotization of the amino group with nitrous acid and coupling with 8-hydroxyquinoline in alkaline medium to give an intense water soluble and stable azo dye with a maximum absorption obtained at a wavelength of 485 nm. The linear calibration curve was applied in the range of (0.5- 50 μg ml^{-1}). The relative standard deviation was estimated as ≤ 1.4% for (30 μg ml^{-1}) chloramphenicol solution of 10 successive injections. This method was found to be easy, simple, reproducible, high precession and very sensitive for the determination of chloramphenicol in pure form and in pharmaceutical preparations comparing with British pharmacopoeia method.

Keywords: FIA, Spectrophotometer, Zn- redactor, Chloramphenicol, Pharmaceutical preparations.

الخلاصة

تم تقدير كميات ضئيلة من مادة الكلورامفينيكول داخل المستحضرات الصيدلانية ضوئياً وبأستخدام التحليل الحقن الجرياني. تعتمد الطريقة على أختزال مجموعة النايترو في الكلورامفينيكول إلى مجموعة الأمين- و بأستخدام عامود الأختزال المعبأ بـ ملغم الخارصين ومن ثم تحويل المادة المختزلة إلى ملح الـ دايزونيوم بواسطة حامض النتروز، وأقتران الأخير مع الكاشف العضوي 8-هايدروكسي كوين- ولين فـي الوسـط القاعدي لتكوين مركب تعتمد شدة لونه على كمية الكلورامفينيكول الموجود في المحلـول المـائي حيـث أظهرت أعلى امتصاصية عند اعظم طول موجي 485 نانوميتر. أنطبق قانون بير-لامبرت على مـدى

التراكيز تتراوح بين 0.5-50 مايكروغرام / مللتر حيث كان الأنحراف القياسي النسبي أقل أو يـساوي 1,4% للتركيز 30 مايكروغرام/مللتر.....أن الطريقة بسيطة وسهلة ودقيقة وحساسة وذات تطابقيـة وتوافقية جيدة ولهذا فقد تم تطبيقها لتقدير الكلورا مفينيكول الموجود بشكل مستحضرات صـيدلانية وتـم تقييم النتائج العملية للطريقة المستخدمة بنتائج الطريقة القياسية المعتمدة من قبل الدستور البريطاني

Introduction

Chloramphenicol (CAP) is a potent inhibitor of bacterial protein synthesis, which was originally isolated from cultures of *Streptomyces venezuelae* [1, 2]. Chloramphenicol remains the drug of choice for the treatment of salmonella infections such as typhoid and paratyphoid fever and in severe systemic Arizona infections. Chloramphenicol is also used in various bacterial eye infections such as *Bacillus cereus panophthalmitis*, which occurs particularly in drug abusers, this is due to its high intraocular penetration properties.

Different methods were described for determination of chloramphenicol in both pure form and as pharmaceutical preparations. Simple photometric and colorimetric determinations of chloramphenicol were investigated [3-5].

The viability of tandem photochemical reaction chemiluminescence's detection was studied for a heterogeneous family of nitro compounds using chloramphenicol as a test substance [6]. Chloramphenicol has also been determined by electro generated chemiluminescence's coupled to flow injection analysis (FIA) [7].

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Recently it has been reported that the antibiotic chloramphenicol has been found in several imported foodstuffs from Asia, including shrimp, crab and crayfish. Several confirmatory analytical liquid chromatography methods with mass spectrometric detection and identification [8] and liquid chromatography- atmospheric pressure photo-ionization mass spectrometry method were developed [9] for the determination of chloramphenicol in shrimps and fish meats respectively.

This work involved the on-line reduction of nitro group of chloramphenicol to amino group using Zn- reductor mini-column, then diazotization of the reduced product with nitrous acid and coupling with 8-hydroxyquinoline (oxine) in alkaline medium, producing an intense water-soluble azo dye with a maximum absorption obtained at a wavelength of 485 nm.

Experimental

Reagents:

All reagents were analytically pure unless stated otherwise and prepared in pure de-ionized water.

The pharmaceuticals tested were chloramphenicol (capsules), samaphenicol (capsules, eye drops) and otocaine (ear drops) from different drug industries. Stock solutions (500 $\mu g\ ml^{-1}$) of chloramphenicol with purity greater than 99% were prepared in ethanol stored in the dark at 4°C, and diluted to the desired concentrations prior to use.

The reductor was prepared according to the Jones reductor [10].

Sodium nitrite (1.0 M): prepared by dissolving 17.25 g of $NaNO_2$ (Fluka) in 250 ml of water and stored in the dark at 4^oC, other working solutions were prepared daily by appropriate dilutions with water.

HCl (1.0 M): 83.3 ml of 36% HCl (BDH) was diluted to 1.0 l with water, other concentrations were prepared by serial dilution.

NaOH (2.0 M): 80 g of the reagent was dissolved in 1.0 l H_2O and working solutions were prepared by appropriate dilution with water.

8- Hydroxyquinoline (1%): 1.0 g was dissolved in 100 ml ethanol.

The chemical tested as foreign compounds in the interference study were soluble starch, KCl, NaCl, $MgCl_2.6H_2O$, sucrose, lactose, glucose, phenol, tetracain and phenyl mercuric acetate (all from Fluka).

Apparatus:

The flow manifold consisted a Rheodyne (5020) injection valve, USA-Haake Buchler MCP 2500 Microprocessor peristaltic pump provided with a Teflon tubes with (0.8 mm i.d.). The flow cell was quartz with a 30 µl volume. The UV- Visible spectrophotometer determinations were accomplished with a model SP8- 300. Fig.(1) shows the diagram of the flow injection manifold.

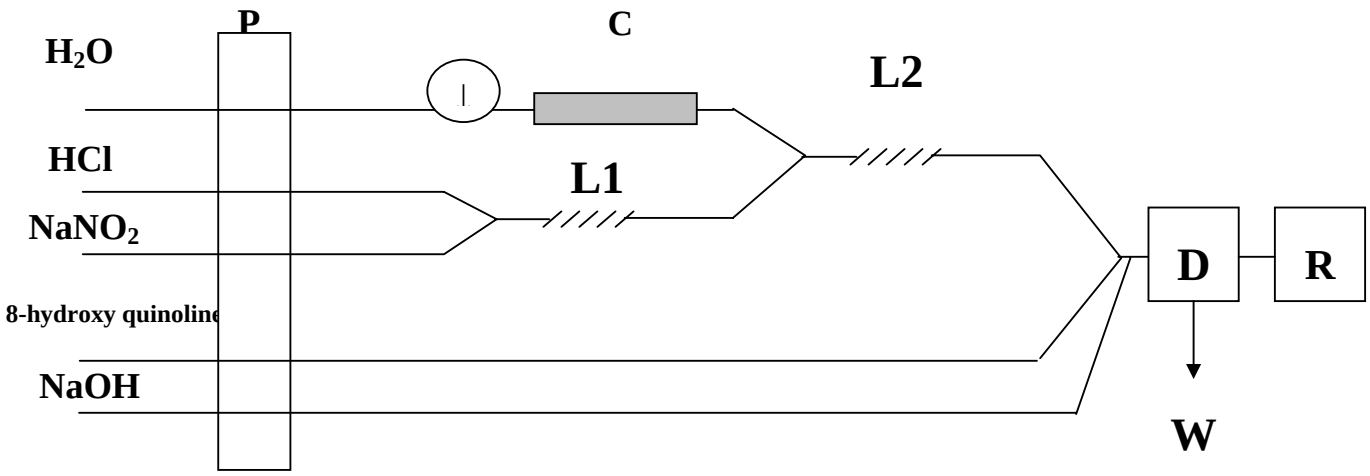

Fig. (1): Flow injection diagram of system for chloamphenicol determination.
P: peristaltic pump, **S:** Sample**, C:** Reductor column**, L1 & L2:** reaction coils**,**
D: Spectrophotometer detector**, R:** Recorder**, W:** Waste**.**

Results and Discussion

Preliminary studies:

First experiments were performed by employing the flow manifold (Fig. (1)) with injecting 100 µl of 500 µg ml^{-1} pure chloramphenicol as a sample and Zn- amalgam packed in a mini-column [10] for reduction of the nitro group of the sample to amino group which diazotized with nitrous acid and coupled with 8-hydroxyquinoline in basic medium, giving an orange color complex with maximum absorption spectrum at a wavelength of 485 nm. The following parameters were selected and taken as bases of the following optimizations, reductor column (2 cm) packed with Zn-amalgam, 0.1 M HCl, 0.1 M $NaNO_2$, 0.1% 8-hydroxyquinoline, 1.0M NaOH, 3.0 ml min^{-1} flow rate, 20 and 100 cm coil lengths for L1 and L2 respectively.

Optimization of FI parameters:

Effect of reductor column length:

The lengths of reductor column were studied from (2- 10 cm) for reduction of 500 µg ml^{-1} of chloramphenicol. Table (1) shows the effect of the reductor column packed with Zn- amalgam on the peak height (mm), in which the optimum length was found to be 5 cm. Zn- amalgam was selected because it is the best reducing agent for chloramphenicol [10, 11].

Table (1): Effect of the reduction column length:

Length of reductor column (cm)	Peak height (mm)
2	58
3	65
4	74
5	77
6	75
7	70
8	67
9	60
10	55

Effect of flow- rate, delay coils and sample volume:

Optimizations of flow rate and delay coils were related to the diazotization of the reduced chloramphenicol and then coupling with 8-hydroxyquinoline. Therefore the manifold in Fig. (1) was operated. A 500 µg ml^{-1} solution of chloramphenicol was selected as a typical example to maintain such physical optimizations. Fig. (2) illustrates the results of the combined flow rate which found that 4.0 ml min^{-1} is satisfactory, because below this flow rate the time is not enough to form complex of the sample with the reagent while at higher flow rate the reduction process of the sample may not be happen completely.

The length of mixing coils was found to be 20 cm (L_1) for mixing 0.1 M HCl with 0.1 M $NaNO_2$ to generate nitrous acids and 90 cm (L_2) to achieve adequate reaction between sample and HNO_2. The sample volume was also optimized and 200 µl found to be the best. Teflon tubing (0.8 mm i.d.) was used for the rest of the manifold.

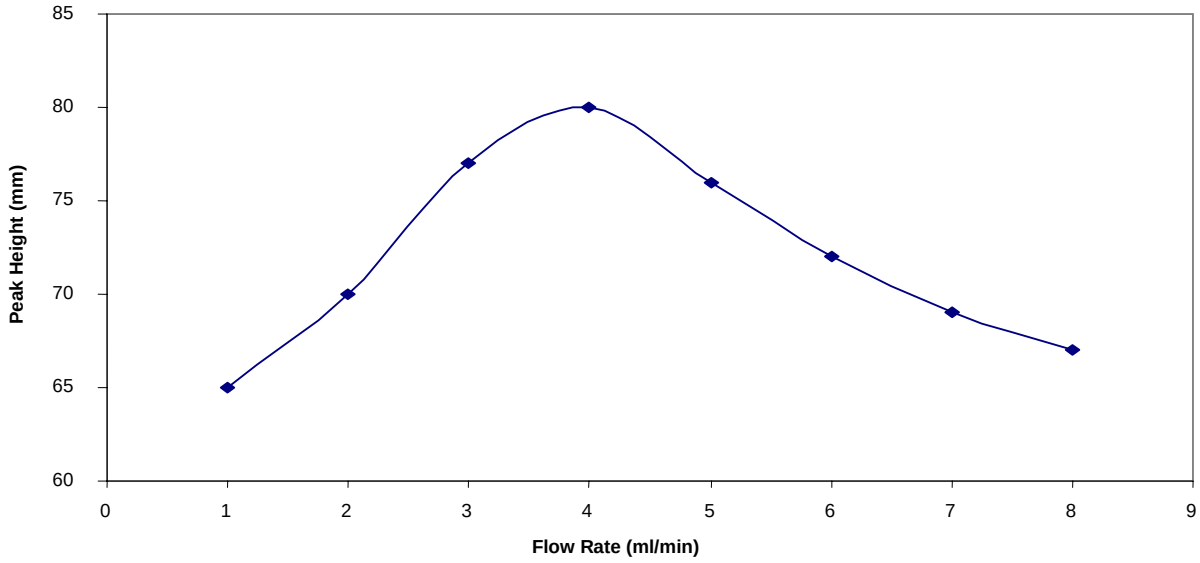

Fig. (2): Effect of flow rate

Optimization of chemical parameters:

Different HCl concentrations were varied over the range 10^{-3}- 1.0 M, its influence on the signals is presented in Fig. (3) (Curve I) a sharp maximum was found at $5x10^{-2}$ M, which this being the concentration used in further work.

The $NaNO_2$ concentration was studied over the range 1.0^{-3}- 1.0 M. Fig. (3) (Curve II) illustrate that 0.1 M gave the best result, which was employed in all subsequent work.

Effect of different concentrations of 8-hydroxyquinoline (0.05- 0.4%) were investigated. Fig. (4) (Curve I) shows that 0.3% resulted in maximum outputs and was selected for further work. On mixing 8-hydroxyquinloine with diazotized chloramphenicol it was found that a color complex is produced only in the presence of strong alkaline medium, therefore different concentrations of NaOH were tested in the range (0.1- 2.0 M). The recorded outputs (Fig. (4) - Curve II) showed that 1.5 M was obtained as the optimum sensitivity.

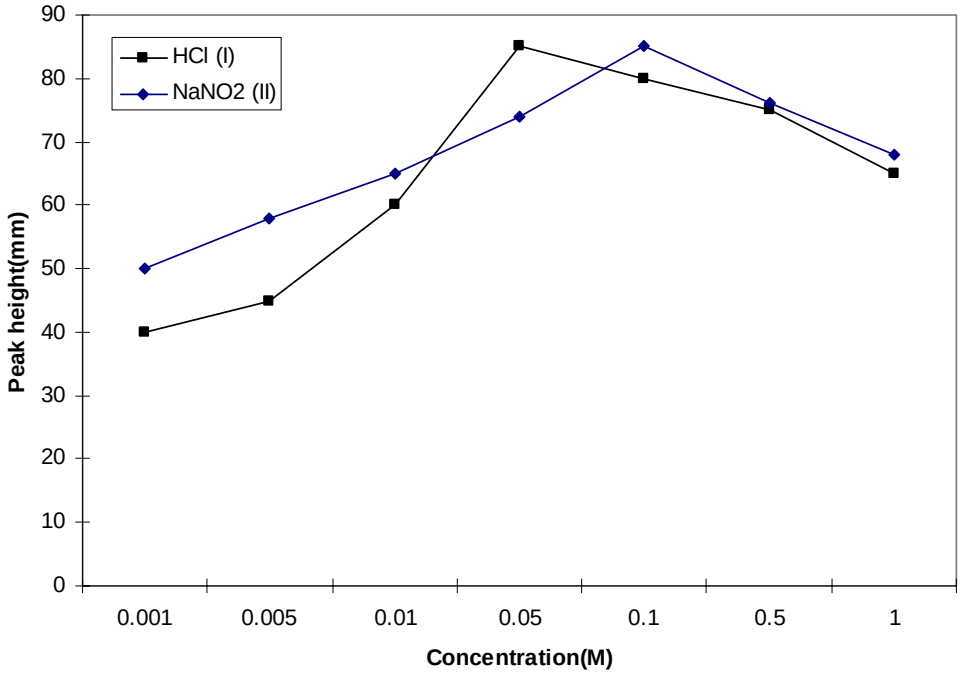

Fig.(3): Signal dependence of (I) HCl; (II) $NaNO_2$ concentrations on the peak height.

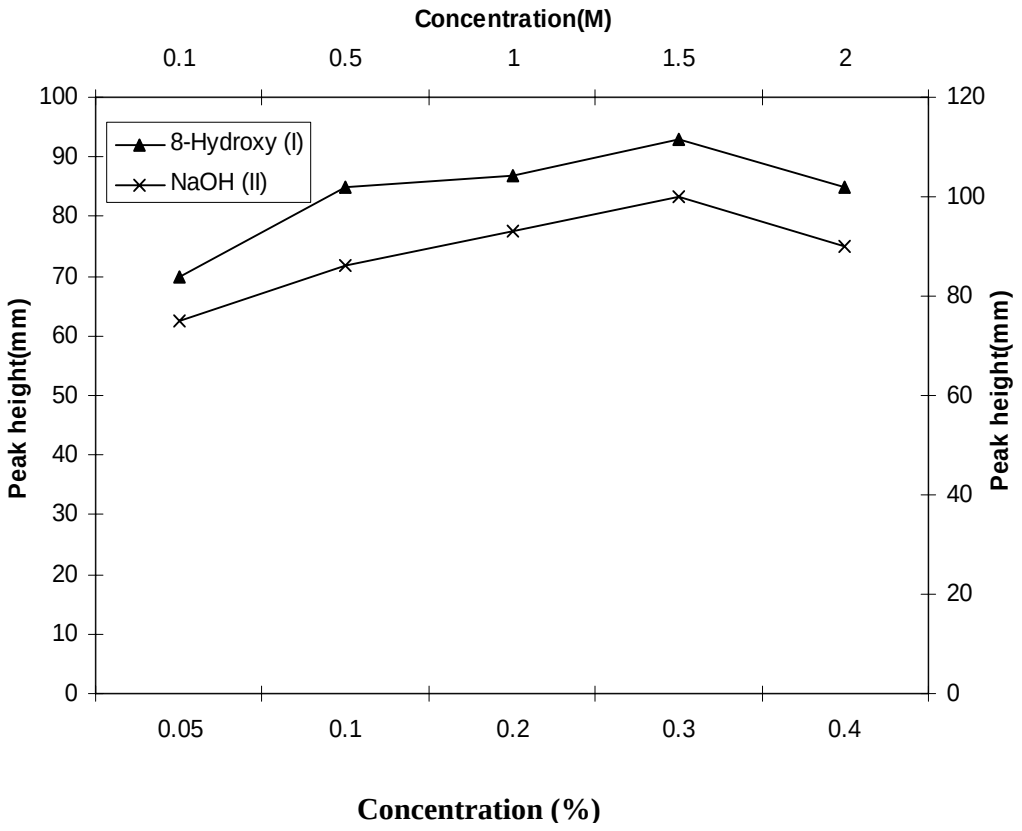

Fig.(4): Signal dependence on (I) 8-hydroxyquinoline; (II) NaOH concentrations on the peak height.

Analytical applications:

After all the parameters were optimized, the calibration curve obtained by using fresh solutions of pure chloramphenicol standards within the range from 0.1- 500 µg ml^{-1}. The obtained analytical calibration curve is described by the equation:

$Y = - 1.05x10^{-2} + 0.0752 \log X$

Where, Y is the peak height in (mm) and X is the chloramphenicol concentration in µg ml^{-1}. The response was linear over the range 0.5- 50 µg ml^{-1} chloramphenicol. The correlation coefficient was estimated as 0.9967.

The limit of detection, defined as the average blank peak height plus 3x RSD, was 10^{-2} µg ml^{-1} of chloramphenicol and was determined experimentally by decreasing the concentration of injected chloramphenicol until this relationship was reached. The relative standard deviation was estimated as ≤ 1.4% for 30µg ml^{-1} chloramphenicol aqueous solution (n= 10) and the sample throughout was $70h^{-1}$.

The response of the chloramphenicol in samples containing other additives such as in pharmaceutical preparations were compared with the response of a solution containing only the pure analyte for a concentration of 10 µg ml^{-1} chloramphenicol, to show the selectivity of the method. Results are shown in Table (2). However, some cationic and anionic interference can be eliminated easily by introduction of a suppressor mini-column into the FIA system [12].

Commercial samples of pharmaceuticals (capsules, eye drops and ear drops) were analyzed by the proposed method and results were compared with the standard methods in British pharmacopoeia [2]. The results are shown in Table (3), and good agreements were found.

Table (2): Effect of foreign compounds on the determination of 10µg ml^{-1} chloramphenicol:

Interfering Compounds	Molar ratio Chloramphenicol/ Interfering	Interference%
NaCl	1:100	+1.00
KCl	1:100	-2.01
$MgCl_2$	1:100	-1.50
Starch	2:10	+1.80
Glucose	1:50	-2.22
Sucrose	1:50	-3.00
Lactose	1:50	+2.03
Phenol	1:50	+2.18
Tetracaine	1:20	+1.89
Phenyl mercuric acetate	1:5	+1.00

Table (3): Determination of chloramphenicol in pharmaceutical preparations:

Drugs	**Contents**	**Amount found**			
		Proposed method	**Recovery%**	**British Pharmacopiea[2]**	**Recovery %**
Chloramphenicol (Capsule)*	250 (mg/capsule)	248 (mg/capsule)	99.2	247.0	98.8
Samaphenicol (Capsule)*	250 (mg/capsule)	251	100.4	251.5	100.6
Samaphenicol (eye drop)*	0.5%	0.51%	102	0.52%	104.0
Otocaine (ear drop)*	1.2%	1.23%	102.5	1.24%	103.3

***Supplier: Massoud-Bahri & Co. "MBC" Damascus- Syria**

Conclusions:

A flow injection spectrophtometric method can be successfully employed for the determination of chloramphenicol in pure form and pharmaceutical preparations with advantages in time of analysis, simplicity, cost, accuracy, precession and sensitivity. The proposed method requires neither temperature control nor solvent extraction step, and can successfully be applied without prelimining treatment. The regeneration of the reductor is simple and not required more than once a week.

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