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Ion-Pair Extraction Spectrophotometric Determination of Trace Amounts of Chloramphenicol

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A simple, sensitive and selective extraction-spectrophotometric method for the determination of trace amounts of chloramphenicol is reported. The chloramphenicol-alizarine yellow ion-pair in acidic media is quantitatively extracted into carbon tetrachloride and its absorbance is measured at 390 nm at room temperature. The effect of different variables such as solvent, volume of extracting solvent, volume of anionic reagent, pH, ionic strength (NaCl 0.1 M) and shaking time is investigated and optimum conditions for quantitative extraction of chloramphenicol is obtained. Linear calibration graph over the chloramphenicol concentration range of 0.03-4.00 μg mL^{-1} and regression coefficient of 0.9999 is obtained. The relative standard deviation of 10 replicate determination of 2 μg mL^{-1} of chloramphenicol is 1.95 %. Limit of detection of the method is 0.022 μg mL^{-1}. The method is used for determination of chloramphenicol in 250 mg capsules and 0.5 % drops and also 150 mg/5 mL of suspension and good results are obtained.

Key Words: Chloramphenicol, Extraction, Alizarine yellow, Determination.

INTRODUCTION

Chloramphenicol is an antibiotic that is clinically useful for serious infections, especially those which result from pneumonia. The majority of the chloramphenicol dose is excreted by the kidneys and due to its pH, it shines above most others antibiotics in terms of ability to penetrate[1].

Various analytical techniques have been reported for the determination of chloramphenicol including UV spectrophotometric, HPLC, mass spectroscopy and LC/MS/MS[2-5]. These methods are either insensitive or inconvenient for topical formulation development with chloramphenicol. A scientillation counting[6] using radiolabeled chloramphenicol formulation is very sensitive and is widely used in the pharmaceutical industry for chloramphenicol formulation development.

Solvent extraction is perhaps the most versatile of analytical techniques, in that it has an extremely wide range of application and invokes most of the physical and

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chemical principles used generally in analytical chemistry[7-9]. It therefore has pedagogic as well as practical value. Extraction methods using ions associated with a large ionic dye or counter ions, forming an ion-associated complex with large molar absorptivities are still limited[10,11]. Many quantitative pharmaceutical determination methods such HPLC suffer from large solvent consumption[12-18]. More investigation in this field could lead to sensitive methods for trace pharmaceutical determinations.

In this paper we intend to examine a simple, sensitive and low cost method for the quantitative extraction of chloramphenicol H^+-alizarine yellow ion pair from an acidic solution into carbon tetrachloride organic solvent followed by spectrophotometric measurements at 390 nm.

EXPERIMENTAL

All the chemicals were of the highest purity available and used without further purification. Double distilled deionized water was used throughout. 20 μg mL^{-1} stock solution of chloramphenicol was prepared by dissolving 0.1 g of chloramphenicol powder in water and diluting to the mark in a 500 mL volumetric flask with distilled water. Working solutions were prepared by appropriate dilution of the stock solution with water. Alizarine yellow, stock solution (3.48×10^{-5} M) was prepared by dissolving proper amount of the dyestuff (Merck) in water and diluting to the mark in a 100 mL volumetric flask. 6 M solution of hydrochloric acid was prepared by diluting 195.5 mL of the concentrated acid to 250 mL in a volumetric flask.

The absorption spectra were recorded on a Shimadzu Model 160A UV-vis recording spectrophotometer was used for absorbance measurements. All pH measurements were made by a metrohm digital pH meter equipped with a combined glass electrode.

Recommended procedure: An aliquot of sample solution containing 0.03-4.0 μg of chloramphenicol was placed in a 10 mL volumetric flask. 2.5 mL of alizarine yellow solution (6.18×10^{-4} M), 0.6 mL of 0.1 M of NaCl solution and 2.0 mL of buffer solution (pH = 4) were added and the solution was diluted to mark with distilled water. The solution was transferred into a 50 mL separatory funnel and 5 mL of carbon tetrachloride was added. The solution was shaken vigorously for 2 min. The phases were allowed to separate and its absorbance was measured at 390 nm against a reagent blank.

RESULTS AND DISCUSSION

Since, chloramphenicol-H^+ cation forms a fairly stable ion-pair with alizarine yellow anionic dyestuff as counter ion in aqueous acidic solution, it is expected to result in a sensitive and useful spectroscopic method for trace chloramphenicol determination.

In preliminary experiments, it was found that the counter anion plays an important role in the formation of a stable ion-pair and among the dyestuffs examined such as methyl orange, orange-II and alizarine yellow, best results were obtained with alizarine yellow dyestuff.

Choice of organic solvent: The extraction process was performed with some common organic solvents such as dichloromethane, chloroform and carbon tetrachloride. It was found that the ion-pair is readily extractable in carbon tetrachloride, while in other solvents used, the coloured complex could not be extracted into the organic phase as completely as carbon tetrachloride.

Effect of alizarine yellow concentration: The effect of alizarine yellow concentration on the extraction of chloramphenicol H^+-alizarine yellow ion-pair was investigated and the results are shown in Fig. 1. It is seen that the absorbance of the organic phase increases with increasing alizarine yellow concentration in the aqueous phase. The concentration 1.54×10^{-4} M of alizarine yellow in the final solution was chosen as the optimum concentration of alizarine yellow.

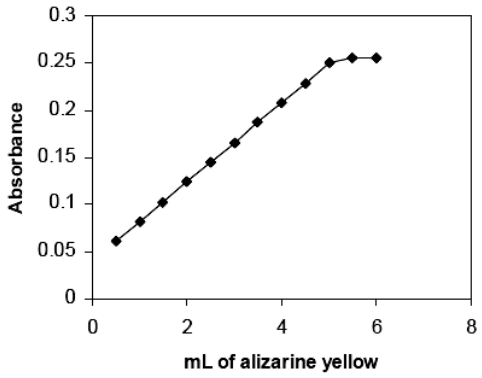

Fig. 1. Effect of alizarine yellow concentration on the absorbance of extracted chloramphenicol H^+-alizarine yellow ion-pair

Effect of buffer solution: The effect of buffer solution on the extraction process of chloramphenicol H^+-alizarine yellow ion-pair was studied using buffer solutions of pH 3-10. On the basis of results presented in Table-1, 2 mL of buffer solution (pH = 4) gives the best results.

TABLE-1
EFFECT OF BUFFER SOLUTION ON THE EXTRACTION OF CHLORAMPHENICOL H^+-ALIZARINE YELLOW ION-PAIR

Buffer solution	Absorbance of extracted ion-pair	Buffer solution	Absorbance of extracted ion-pair
3.0	0.210	7.0	0.046
4.0	0.240	8.0	0.024
5.0	0.167	9.0	0.022
6.0	0.076	10.0	0.011

Effect of solvent volume: It must be mentioned that quantitative extraction of chloramphenicol H^+-alizarine yellow ion-pair is complete by 5 mL of carbon tetrachloride in a single stage extraction process. This was confirmed by observing further enhancement in the absorption of complex in the mixture of two 5 mL portions of the organic phase obtained from two successive extraction of an aqueous chloramphenicol H^+-alizarine yellow ion-pair solution, compared with that of an 10 mL organic phase obtained from a single stage extraction of the same complex solution under optimal experimental conditions. The absorbance reading for 10 mL organic phase containing the chloramphenicol H^+-alizarine yellow ion-pair is half of the absorbance from 5 mL carbon tetrachloride from the first stage of extraction.

Effect of shaking time: The effect of shaking time on the extraction of chloramphenicol H^+-alizarine yellow ion-pair was studied. A shaking time of 2 min was found to be sufficient for the extraction of ion-pair. By increasing the shaking time, the absorbance of related blank will have no difference.

Analytical performance: Under the optimum conditions described above. Linear calibration graph for chloramphenicol was obtained in the concentration range of 0.03-4.0 μg mL^{-1}. The regression equation for chloramphenicol is $A_{chloramphenicol} = 0.0613C_{chloramphenicol} + 0.0044$ (where $A_{chloramphenicol}$ is the absorbance of the sample against a blank solutions and $C_{chloramphenicol}$ is the concentration of chloramphenicol in μg mL^{-1}) with a correlation coefficient of 0.9999. The relative standard deviation (RSD) of 10 replicate determination of 2.0 μg mL^{-1} of chloramphenicol is 1.945 %, respectively and the limit of detection (LOD)[19] of the method is 22 ng mL^{-1}.

Effect of interferences: In order to study the influence of various cations and anions on the determination of chloramphenicol, a fixed concentration of chloramphenicol, 2.0 μg mL^{-1}, was taken with different amounts of foreign ions and the recommend procedure was followed. A relative error of ± 5 % with respect to the absorbance difference for the chloramphenicol solution was considered tolerable. Tolerance limits are as follows: Cu^{2+}, Li^+, NH_4^+, I^- (1200 folds), NO_3^-, Fe^{2+}, Fe^{3+}, Na^+, Ca^{2+}, K^+, SCN^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-} (1000 folds), Mn^{2+} (900 folds), Pb^{2+}, Ni^+, Co^{2+}, Mg^{2+}, Cl^- (800 folds). The results show that most of the cations and anions used have no considerable effect on the determination of chloramphenicol.

Application: The proposed method was applied to the determination of chloramphenicol of 250 mg capsules and in 0.5 % drops and also in 150 mg/5 mL of suspension, (from Chemi Daruo Co). There is a satisfactory agreement between the results of the proposed method and the reference value of minoxidil content of the formulations (Table-2).

TABLE-2
DETERMINATION OF THE CHLORAMPHENICOL CONTENT OF 250 mg FORMULATION CAPSULES, 0.5 % DROPS AND 150 mg/5 mL SUSPENSION BY THE PROPOSED METHODS

Sample	Reference amount (mg)	Obtained (mg)	Recovery (%)
1 (Capsules)	2.0	1.961	98.05
2 (Drops)	2.0	1.999	99.95
3 (Suspension)	2.0	1.978	98.90

Conclusion

The method described, provides a simple and reliable means of determination of trace amounts of chloramphenicol in formulation samples. The limit of detection of the proposed method seems to be good. The method compares favourably in sensitivity and selectivity with most of the published methods[20-23] for the determination of chloramphenicol and it can certainly be placed among the most sensitive ones.

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