

## Spectrophotometric Determination of Chloramphenicol in Pure and Pharmaceutical Formulations

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\*A simple and sensitive spectrophotometric method has been developed for the determination of chloramphenicol (CAP) in bulk and in pharmaceutical formulations. The proposed method involves reduction of the analyte with Zn-HCl followed by diazotisation and coupling of the resulting diazonium salt with resorcinol in alkaline medium to form a coloured chromogen. The results of analysis of this method have been validated statistically and found to be precise and accurate.

### INTRODUCTION

Chloramphenicol (CAP, D(-)-threo-2-dichloro acetamide-1-*p*-nitrophenyl-1,3-propanediol), a broad spectrum antibiotic, was first isolated by Ehrlich *et al.*<sup>1</sup>, from cultures of *Streptomyces venezuelae*. It is commercially available in capsules, tablets, oral suspensions and eye ointments. It is often used in veterinary practice for treating various infectious diseases. Nevertheless, adverse reactions and side effects in humans have been extensively demonstrated. Problems related to chloramphenicol use have been revised<sup>2</sup>. Because chloramphenicol is used for therapeutic and prophylactic purposes in veterinary medicine, especially for the treatment of mastitis in cattle, and because toxicological problems such as gray syndrome and aplastic anemia have occurred in humans exposed to this antibiotic, a health hazard to humans may result from eating animal derived foods such as milk or meat that contain chloramphenicol residues. Infant fatalities owing to chloramphenicol administration have also been reported<sup>3-5</sup>.

Chloramphenicol (CAP) uses, toxicity and chromatographic methods have been reviewed<sup>6-14</sup>. CAP has been spectrophotometrically determined by reaction between its alkaline hydrolysis products and ammonium molybdate or by formation of their azo derivatives<sup>15</sup>; it has also been determined by reaction with cobaltinitrite<sup>16</sup>. Other photometric methods used are based on reduction of its nitro group, followed by diazotisation and coupling<sup>17</sup>. Hassan *et al.*<sup>18</sup> determined CAP and its esters in pharmaceutical formulations by reduction of the NO<sub>2</sub> group with cadmium in acid medium followed by determination of the cadmium ions

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produced. Polarography<sup>19-21</sup> and atomic absorption spectrometry<sup>22, 23</sup> have also been used for the determination of CAP.

This paper describes a procedure for the determination of chloramphenicol. The method involves reduction of the nitro compounds to the corresponding primary aromatic amines followed by diazotisation of the latter and coupling of the resulting diazonium salts with resorcinol. The method is simple, selective, sensitive and readily adaptable to the analysis of pharmaceutical formulations.

## EXPERIMENTAL

A Hitachi, model U-2000, double beam spectrophotometer with 1 cm (quartz) cells was used for measurements of absorbance.

All reagents and chemicals used were of AR grade. Aqueous solutions of resorcinol 0.25% (w/v), sodium hydroxide 1 N, hydrochloric acid 5 N, sodium nitrite 0.5% (w/v), zinc powder, methanol and saturated solution of sulphamic acid were prepared in the usual way.

**Preparation of Standard Drug Solution:** Stock solution of chloramphenicol (1 mg/mL) was prepared in methanol. From this, working standard solutions are prepared by suitably diluting the stock solutions to obtain a concentration of 100 µg/mL.

### Preparation of Sample Solutions

**Tablets and Capsules:** Two or three tablets or the contents of 2 or 3 capsules were placed in a mortar and ground to a fine powder. An amount of powder equivalent to 25 mg of pure CAP was dissolved in 50 mL of methanol, filtered and diluted to 250 mL with distilled water to obtain a working solution of 100 µg/mL.

**Eye Ointment:** An accurately weighed portion of ophthalmic ointment equivalent to about 10 mg of pure CAP was shaken in a separating funnel with about 50 mL of n-hexane and then extracted with four 20 mL portions of water. The aqueous extracts were combined in a 100 mL standard flask and diluted to volume with methanol to obtain a working solution of 100 µg/mL.

**Recommended Procedure:** Aliquots of standard chloramphenicol solution 0.5–1.5 mL (100 µg/mL) were placed in a series of 10 mL volumetric flasks followed by adjusting the volume to the mark with methanol and absorbance was measured at  $\lambda_{\max}$  271 nm against the blank.

Transfer 5 mL of 1 mg/mL standard drug solution into a 50 mL volumetric flask, add 10 mL of water, 5 mL of hydrochloric acid and 250 mg of zinc powder. Allow the flask to stand for 15–30 min, then make up to the mark with distilled water and filter the solution into a dry flask. Transfer suitable volumes of this solution into 25 mL volumetric flasks to obtain the working concentration ranges. Aliquots of the sample solution 0.5–2 mL (100 µg/mL) are taken into 10 mL volumetric flasks. Then add 0.5 mL of sodium nitrite reagent and after 5 min, add 1 mL of sulphamic acid solution; allow to stand for 5–10 min, then add 1.5 mL of resorcinol solution followed by 5 mL of 5 N sodium hydroxide solution and dilute to volume with distilled water. The absorbance is measured at 375 nm

after 5 min against a reagent blank prepared in a similar manner. The absorbance is plotted against the final concentration to obtain a calibration graph.

## RESULTS AND DISCUSSION

Chloramphenicol is a nitro derivative in which the nitro group is attached to an aromatic moiety; on reduction with Zn-HCl, it yields the corresponding primary amine which, after diazotisation, can be coupled with resorcinol in a strongly alkaline medium to produce red coloured complex. This reaction is utilised for the spectrophotometric determination of chloramphenicol in pure and pharmaceutical formulations. The coupling reaction is instantaneous and the colour remains perfectly stable for more than 24 h.

It has been observed that methanolic solution of unreduced standard chloramphenicol exhibits an absorbance maximum of 271 nm. The coloured complex derivative obtained by the proposed method shows maximum absorbance at 375 nm. Various common organic solvents have been tried for the extraction of the chromophore. The derivative has been found not to be extractable with common organic solvents. An additional advantage is that the coloured products are soluble in alkalis; hence, no precipitation occurs, and tedious extraction steps are not necessary.

Analytical parameters, Beer's law range, molar absorptivity and Sandell's sensitivity are given in Table-1 along with the slope and correlation coefficient obtained from the regression equation using the least-squares method. When pharmaceutical formulations containing chloramphenicol are analysed, the results obtained by proposed method are given in Table-2 with good agreement in labelled amounts. In order to confirm the reliability and suitability of the proposed method recovery studies have been carried out by adding to known quantities of standard drug solution previously analysed samples and re-analysing the same by the proposed method. The recovery is found to be 98.40–100.08. The results compare favourably with those of the official methods<sup>24, 25</sup>.

TABLE-1  
ANALYTICAL AND STATISTICAL PARAMETERS OF  
CHLORAMPHENICOL USING PROPOSED METHOD

Parameter	Value
$\lambda_{\max}$	375
Beer's law limit ( $\mu\text{g/mL}$ )	0.50
Molar absorptivity ( $\text{lit mole}^{-1} \text{cm}^{-1}$ )	$8.45 \times 10^2$
Sandell's sensitivity ( $\mu\text{g/cm}^{-2}$ )	0.0526
Slope	0.9965
Correlation coefficient	0.9986

TABLE-2  
RECOVERY STUDY DATA

Formulations*	Amount labelled	Amount spiked (mg)	Recovery %†	
			Proposed method	Official method <sup>24, 25</sup>
Capsules	500 mg/caps	25	100.4	100.37
Capsules	250 mg/caps	25	99.9	-
Tablets	250 mg/tab	25	98.4	99.00
Tablets	250 mg/tab	25	100.6	-
Ointment	1000 mg/100g	10	100.8	99.30
Ointment	10 mg/g	10	99.7	99.50

\*Formulation from different manufacturers

†Average of three determinations

No interference was observed due to diluents, excipients and colour present in the dosage form. The proposed method is simple, accurate and reproducible and can be used for routine analysis of chloramphenicol in bulk drug and in its formulations.

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### REFERENCES

1. J. Ehrlich, Q.R. Bartz, R.M. Smith and D.A. Joslyn, *Science*, **106**, 417 (1947).
2. E.H. Allen, *J. Assoc. Off. Anal. Chem.*, **68**, 990 (1985).
3. W.L. Thompson, *J. Am. Med. Ass.*, **234**, 149 (1975).
4. E. Grusz-Harday, *Bull. Int. Ass. Forens. Toxicol.*, **9**, 10 (1973).
5. H. Kucers, *J. Antimicrob. Chemother.*, **6**, 1 (1980).
6. K.P. Holland, A.C. Henry, R.T. Wilson and J.S. Dreas, *J. Assoc. Off. Anal. Chem.*, **78**, 483 (1995).
7. R.I. Epstein, A.C. Henry, K.P. Holland and J.S. Dreas, *J. Assoc. Off. Anal. Chem.*, **77**, 570 (1994).
8. R.M.L. Alerts, H.J. Keukens and G.A. Werdmuller, *J. Assoc. Off. Anal. Chem.*, **72**, 570 (1989).
9. P. Sanders, P. Guillot, M. Dagorn and J.M. Delmas, *J. Assoc. Off. Anal. Chem.*, **74**, 483 (1991).
10. J.P. Abjean, *J. Assoc. Off. Anal. Chem.*, **80**, 737 (1997).
11. Javier Bayo, M.A. Moreno, Javier Prieta, Susana Diaz, Guillermo Suarez and Lucas Dominguez, *J. Assoc. Off. Anal. Chem.*, **77**, 854 (1994).
12. H.J. Keukens, M.M.L. Aerts, W.A. Traag, J.E.M. Nouws, W.G. DeRing, W.M.J. Beek and J.M.P. Den Hartog, *J. Assoc. Off. Anal. Chem.*, **75**, 245 (1992).

13. M.F. Pochard, G. Burger, M. Chevalier and E. Gleizes, *J. Chromatography*, **409**, 315 (1987).
14. A. El-Yazigi, A. Yusuf and Al-Humaiden, *Clin. Chem.*, **33**, 1814 (1987).
15. M.E. Abdel-Hamid and M.A. Abuirjeie, *Analyst*, **112**, 895 (1987).
16. M.S. Mahrous and M.M. Abdel-Khalek, *Talanta*, **31**, 289 (1984).
17. F. Snell and C. Hilton *Encyclopedia of Industrial Chemical Analysis*, Interscience, New York, Vol. 5 (1974).
18. S.S.M. Hassan and M.H. Eldesouki, *Talanta*, **26**, 531 (1979).
19. K. Kannan, R. Manavalan and A.K. Kelkar, *Indian Drugs*, **25**, 128 (1987).
20. S. Seth and N.R. Banerjee, *Indian J. Pharm. Sci.*, **49**, 58 (1987).
21. A. Morales, M.I. Toral and P. Richter, *Analyst*, **109**, 633 (1984).
22. T. Mitsui and T. Kojima, *Bunseki Kagaku*, **26**, 317 (1977).
23. R. Montero, M. Gallego and M. Valcarcel, *Talanta*, **37**, 1129 (1990).
24. *British Pharmacopoeia*, H.M. Stationary Office, London, Vol. I & II (1980).
25. *United States Pharmacopoeia*, 21st Revision, Rockville, MD, p. 760 (1985).

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