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Spectrophotometric assay for chloramphenicol and some derivatives in the pure form and in formulations

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Abstract: A new analytical method for chloramphenicol and its derivatives, chloramphenicol succinate sodium and D-(−)-threo-2-amino-L-[*p*-nitrophenyl]-1,3-propanediol (chloramphenicol base) in the pure state or in pharmaceutical preparations, is described. The method is based on measuring the blue colour produced by the interaction of ammonium molybdate with the products of the alkaline hydrolysis of the drugs. There are certain advantages of simplicity and sensitivity over current methods. The Beer's law limits, effects of temperature, acidity and reagent concentration, and statistical analysis of experimental results are reported.

Keywords: *Chloramphenicol assay; chloramphenicol succinate sodium; pharmaceutical formulations; visible spectrophotometry.*

Introduction

Chloramphenicol, D-(−)-threo-2-dichloracetamido-L-[*p*-nitrophenyl]-1,3-propanediol (CP) and its esters are broad-spectrum antibiotics of widespread therapeutic application. The assay of these drugs has been, and is still the subject of much investigation. Methods based on titrimetry [1, 2], chromatography [3, 4], polarography [5, 6], atomic absorption spectrometry [7], potentiometry [7] and spectrophotometry [7–12] have been described. However many of these assay methods are limited in their applications or are rather tedious and time-consuming. There is therefore a need for a simple spectrophotometric method for the assay of CP and its esters.

Spectrophotometric methods for determining cephalosporins [13] and tetracyclines [14] have been described based on their oxidation with ammonium molybdate. As part of a continuing investigation on analytical procedures for antibiotics the reduction of molybdate to molybdenum blue by CP was also investigated. It was discovered that CP, chloramphenicol succinate sodium (CPS) and a compound structurally related to CP, D-(−)-threo-2-amino-L-[*p*-nitrophenyl]-1,3-propanediol (chloramphenicol base CPB), after alkaline hydrolysis, produced a blue colour with ammonium molybdate in sulphuric acid medium. CP and its derivatives undergo hydrolysis in strongly alkaline medium to give products, the infrared spectra of which indicate the presence of a free amino group. The intensity of the blue colour varied with amount of antibiotic added. A systematic study was therefore carried out to investigate the variables affecting the reaction between

these drugs and molybdate, to optimize the conditions, and to develop a simple and inexpensive spectrophotometric procedure that can be adapted easily for the determination of CP, CPB and CPS. The method that has been developed is sensitive and precise and has been used for the assay of drug formulations.

Experimental

Reagents and equipment

The drug standards. Chloramphenicol, chloramphenicol base and chloramphenicol succinate sodium were obtained from Sigma Chem. Co. (USA). The drug formulations, Chemycetin succinate injections (containing CPS) and Chemycetin capsules (containing CP), manufactured by Farmitalia Carlo-Erba (Italy), were obtained locally.

Preparation of drug solutions. Weigh 12.5 mg of CP, CPB or CPS accurately into a 50-ml conical flask containing 20 ml of 4 M sodium hydroxide. Loosely stopper the flask and heat for 5 min, while stirring. Cool thoroughly and adjust to volume with 4 M sodium hydroxide in a 25-ml calibrated flask. The resulting concentration is 0.5 mg/ml.

Sulphuric acid. 50% v/v solution.

Ammonium molybdate. 25% m/v in 50% v/v sulphuric acid solution. Dissolve, by stirring, 6.25 g of ammonium molybdate in about 20 ml of 50% v/v sulphuric acid solution in a 25-ml calibrated flask, then dilute to volume with the same solvent.

Spectrophotometer. A Perkin–Elmer 555 spectrophotometer equipped with 1-cm quartz cells was used. All measurements were made in the double-beam mode; automatic baseline correction was employed, the baseline being determined with both sample and reference cuvettes filled with reagent blank solution.

General procedure

Pipette a volume of drug standard solution expected to contain up to 100 μg of compound into a 5-ml calibrated flask. Add 2.5 ml of 25% m/v ammonium molybdate solution and then 2 ml of concentrated sulphuric acid. Cool to room temperature and dilute to the mark with distilled water. Mix well, heat to 95°C in a constant temperature bath for 50 min, and then adjust the solution to room temperature briefly cooling in ice-water to quench the reaction. Measure the absorbance at 670 nm against a reagent blank prepared under the same conditions.

Procedure for injections

Extraction of CPS from Chemycetin succinate powder for injections was found to be unnecessary. Prepare working solutions of the drug as previously described for the antibiotic standards then perform the determination as described under the section "General procedure".

Procedure for capsules

With formulated preparations, it is necessary first to isolate the active ingredient from the other ingredients. This is accomplished by extracting the CP by a suitable solvent, as follows.

Grind the contents of three Chemycetin capsules containing 250 mg CP in each capsule in a mortar. Weigh an appropriate quantity of the powder equivalent to 500 mg of the drug in a 250-ml conical flask and swirl for about 10 min with *ca* 200 ml of ethanol. Remove the insoluble components by filtration and evaporate the filtrate to dryness under vacuum. Prepare sample solutions after alkaline hydrolysis of accurately weighed portions of the drug residue and continue as described under the section "General procedure". Determine the CP content in the formulations either from a calibration graph or a regression equation for the appropriate standard antibiotic.

Results and Discussion

Preliminary studies were carried out to determine the conditions for the development of maximum colour of the reaction product.

Effect of temperature

The absorbance was measured at 670 nm of samples prepared as previously described using various temperatures and times of heating. The time and temperature of heating was found to affect the sensitivity of the reaction; heating for 50 min at 95°C was found to be the most suitable condition for the completion of the reaction. Higher temperatures and longer heating times had no apparent advantage. Lower temperatures reduced the reaction rate, which at room temperature was impracticably slow.

A set of graphs obtained with samples of 18 μg/ml of CPB is shown in Fig. 1. Similar results were obtained with samples of both CP and CPS.

Effect of concentration of sulphuric acid

Figure 2 shows the graphs of absorbance versus sulphuric acid concentration. The absorbance at 670 nm increases with increasing sulphuric acid concentration up to 11 M then remains almost constant.

Effect of concentration of reagent

The effect on the absorbance of varying the volume of the 25% m/v molybdate solution from 0.5 to 2.7 ml is shown in Fig. 3. The highest absorbance was obtained by

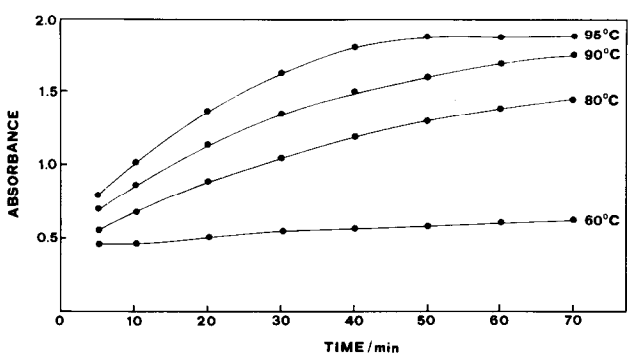

Figure 1
Effect of temperature and heating time on absorbance of the coloured product. 18 μg/ml of chloramphenicol base; 670 nm; reference, reagent blank.

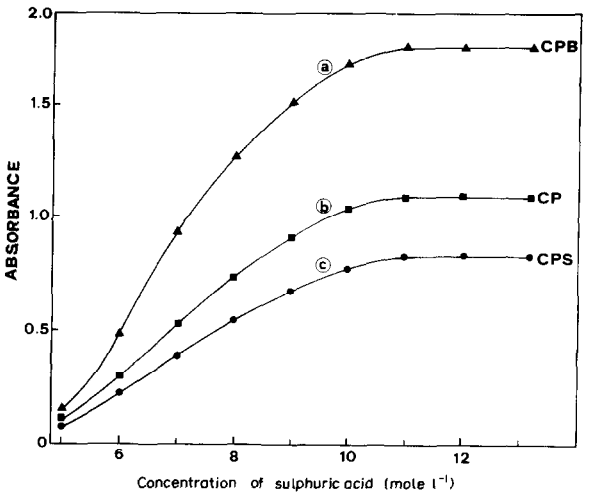

Figure 2
Effect of sulphuric acid concentration. (a) 17.6 μg/ml of chloramphenicol base; (b) 14.3 μg/ml of chloramphenicol; (c) 13.3 μg/ml of chloramphenicol succinate sodium. 670 nm; reference, reagent blank.

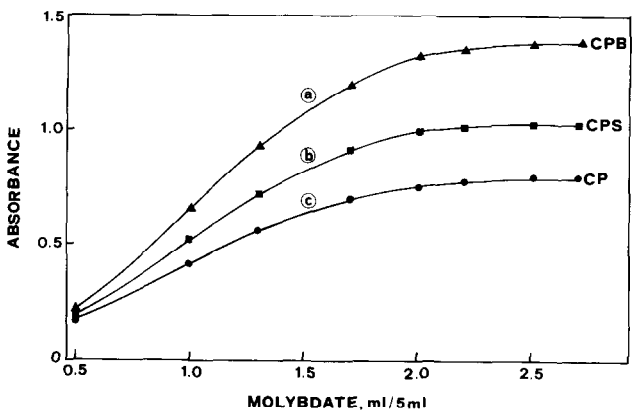

Figure 3
Effect of the reagent concentration. (a) 13.4 μg/ml of chloramphenicol base; (b) 15.8 μg/ml of chloramphenicol succinate sodium; (c) 9.9 μg/ml of chloramphenicol. 670 nm; reference, reagent blank.

using 2.5 ml of the 25% molybdate solution; above this volume the absorbance remains constant. A volume of 2.5 ml was therefore used in all further measurements.

Absorption spectra

The spectra recorded under the specified conditions of the assay exhibited a maximum at 670 nm which was attributed to molybdenum blue formed by the reduction of molybdenum (VI) in the presence of the hydrolysed drugs. The blank solution shows a small absorbance around this wavelength and this indicates the need for measurements to be performed against a reagent blank.

Calibration graphs and statistical analysis

Several standard solutions of CP, CPB and CPS at concentrations up to 20 μg/ml were assayed by the molybdate spectrophotometric method. Table 1 shows the results of the statistical analysis of the standard calibration curves of each drug.

Beer's law was obeyed up to 20 μg ml^{-1}. The molar absorptivities of CP, CPB and CPS at 670 nm, calculated from the linear regression data, were found to be 2.6×10^4, 2.2×10^4 and 2.8×10^4 l mol^{-1} cm^{-1}, respectively. The Sandell's sensitivities were calculated to be 0.0124, 0.0096 and 0.0152 μg/cm^2 per 0.001 absorbance unit, respectively. The differences among the molar absorptivities of CP, CPB and CPS, may be related to the different chemical structures of these compounds. Ammonium molybdate is strongly reactive under the experimental conditions, and it may react to different extents with the decomposition products of the three drugs, giving the small differences observed.

Table 1
Statistical analysis* of the spectrophotometric assay of chloramphenicol and its derivatives

Compound	*Y*-Intercept	Correlation coefficient	Slope (cm^{-1} μg^{-1} ml)	Variance (s_o^2)	Detection limit† (μg/ml)
Chloramphenicol	0.001	0.9996	0.0806	1.05×10^{-4}	0.37
Chloramphenicol base	0.004	0.9996	0.1045	1.80×10^{-4}	0.37
Chloramphenicol succinate sodium	0.003	0.9997	0.0658	5.80×10^{-4}	0.34

*Number of standard solutions, $n = 15$.
†Level of significance, $p = 0.01$.

The detection limit (DL) for the method was calculated by means of the following relationship [15]:

$$DL = \sqrt{S_o^2 \frac{n-2}{n-1} \frac{t_p}{b}},$$

where: n = number of samples; b = slope of line of regression; t_p = Student's t value at $p = 0.01$ level of significance; S_o^2 = variance = $\Sigma(A - A_{calc})^2/n - 2$ [16] (A = experimental value of absorbance; A_{calc} = absorbance value calculated from the regression equation).

The good sensitivity of the method is indicated by both the detection limits and the slopes of the calibration graphs. The small degree of scatter of the experimental data points around the line of regression is confirmed by the small values of variance.

Samples of Chemycetin capsules (250 mg) and Chemycetin injectable dosage forms containing 1 g CPS/vial, were assayed as described in the Experimental section. Ten replicate determinations of each sample solution were carried out to test the accuracy and precision of the method. The standard deviations (SD) and relative standard deviations (RSD) were; Chemycetin capsules, SD = 0.56 and RSD = 1.21%; Chemy-

cetin succinate injections, SD = 0.42 and RSD = 1.22%. These results indicate that the method has satisfactory reproducibility.

Statistical analysis of the calibration data allows the calculation of the error (S_c) in the determination of a given concentration (c) by means of the equation [16]:

$$S_c = \frac{S_o}{b}\left(1 + \frac{1}{n} + \frac{(A - A')^2}{b^2\,(\Sigma c^2 - nc'^2)}\right)^{1/2},$$

where c' and A' = average concentration and absorbance values, respectively, for n standard solutions.

Figure 4 shows the graphs of S_c versus the final concentrations of CPB (curve a), CP (curve b) and CPS (curve c). The error is at a minimum for $A = A'$, corresponding to about 8 μg/ml.

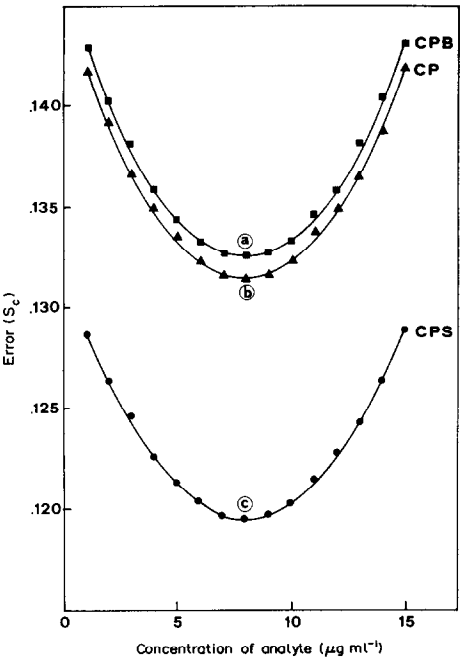

Figure 4
Error in the determination of the concentration of (a) chloramphenicol base, (b) chloramphenicol and (c) chloramphenicol succinate sodium, obtained by statistical analysis of standard calibration data.

Conclusions

The molybdate method represents a useful procedure for determining chloramphenicol and its derivatives in the drug substances and in certain formulations. The procedure is very simple and may be carried out at low cost as it involves few reagents and reaction stages. The sensitivity is sufficiently high and compares favourably with that of other known methods. The accuracy and reproducibility of the results are very satisfactory.

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