THE ASSAY OF CHLORAMPHENICOL IN PHARMACEUTICAL PREPARATIONS BY MEANS OF A SIMPLE COUNTER CURRENT TECHNIQUE*

By Arne Brunzell

From the Chemical Department of the State Pharmaceutical Laboratory, Stockholm, Sweden

Received December 5, 1955

Chloramphenicol in a pure form can be satisfactorily characterized by chemical and physical examination. In pharmaceutical preparations it is generally considered that microbiological assay is the only possible method. That there are possibilities of eliminating the microbiological assay for the careful control of some of the usual chloramphenicol preparations is here shown.

In the monographs in the B.P. and Ph.I. the substance is assayed by the absorption of light at 278 mμ and identified by tests for organically bound chlorine, reaction of the nitro group, the melting point and optical activity. In the regulations published by the Food and Drug Administration, Washington, D.C., a microbiological assay is given as an alternative method. The U.S.P. XIV referred to these regulations, thus accepting both assays, but the U.S.P. XV has adopted only the microbiological method.

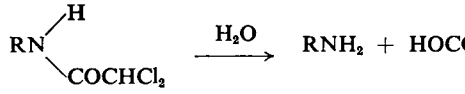
Quantitative determinations of chloramphenicol in preparations are included in the B.P. (capsules) and in the U.S.A. Federal Register (capsules, ointment, ophthalmic, injection, otic and tablets). The B.P. method for capsules is a gravimetric one after extraction with ether. The Federal Register uses the microbiological method, after extraction with water, for all preparations but gives as an alternative for capsules, eye drops and tablets the spectrophotometric determination of an aqueous extract. No test for decomposed chloramphenicol is included in any of these regulations.

Other quantitative non-official methods for the assay of chloramphenicol as a substance or as an ingredient in preparations have been published. They make use of those chemical properties of the chloramphenicol molecule which make possible the determination without isolation. The reduction of the nitro group is the principle of a polarographic determination[1]. After the quantitative reduction of the nitro group the amino group formed is titrated[2] or diazotised for final photometric determination[3]. The dichloroacetic acid part of the molecule is determined by titrimetric[4] and photometric methods[5]. The antibiotic may be quantitatively oxidized with periodic acid after hydrolysis[6], or the yellow colour occurring when it is heated with alkali may be used for the photometric determination in solutions[7].

* The main results of this paper were reported by H. Hellberg at the meeting of the Directors of Control Laboratories at the 16th General Assembly of the F.I.P. in London, in September, 1955.

The isolation from fermentation broths is described by Bartz[8], who also reports some distribution ratios between water and organic solvents, and the estimation in biological materials is described by Glazko *et al.*[3]

Chloramphenicol is rather easily hydrolysed mainly according to the reaction



with dichloroacetic acid and 1-*p*-nitrophenyl-2-amino-1 : 3-propandiol as the products[9]. This primary amine is microbiologically almost inactive[10] but is easily included in a chemical assay. This seems to be the reason for the discrepancy between chemical and microbiological assays on some chloramphenicol preparations reported by Dony[11]. The ultra-violet absorption at 278 mμ is so little changed that decomposition would not be noticed without a wavelength control of the maximum. The total hydrolysis changes the maximum only 7 mμ, from 278 mμ to about 271 mμ[10]. As the nitro group is left unchanged by the hydrolysis, determinations using that group may also be unreliable.

The method of separating chloramphenicol from its degradation products in biological materials has been published by Glazko *et al.*[3] and others.

To be able to determine chloramphenicol unambiguously and to distinguish it from, for example, the optical isomers, a chemical method must start with an isolation followed by determination of melting point and optical activity since the isomers differ in these properties[12]. This approach may be used for many preparations because of the solubility of chloramphenicol. If in addition hydrolysis is suspected the isolation must include a separation from the decomposition products.

This report will show that it is possible to use a chemical assay to a greater extent than existing regulations permit.

EXPERIMENTAL

Sometimes simple extractions, such as described in the B.P. for the assay of chloramphenicol in capsules, may be sufficient. Otherwise by applying simple counter current extractions systematically in the way earlier described by Brunzell and Hellberg[13] it is possible to isolate and characterize the drug from mixtures having the most diverse constituents. This technique requires knowledge of the distribution ratios, as these are used in the calculation of the separation procedure. For this purpose the ratios in Table I were determined.

The solubility in water is low but sufficient, and the distribution ratios are very different, some much larger than unity, some much smaller. Thus it is possible to arrange suitable separation procedures. Moreover the distributions are as a rule not much influenced by the pH of the aqueous phase, a fact that makes the planning easier. However, Bartz[7] shows some variations for the systems with benzene and carbon tetrachloride at the pH values 2·15, 6·50 and 9·00.

When trying to obtain the free amine together with chloramphenicol a noticeable decomposition of the amine seems to occur, thus colouring the substance yellow to brown.

The partition properties of the primary amine are quite different, however, see Table II. On distributing material between equal volumes of ethyl acetate and water at pH 7 more than 90 per cent. of the amine will go into the water phase but only 3 per cent. of the chloramphenicol.

TABLE I

DISTRIBUTION RATIOS FOR CHLORAMPHENICOL

Water/ethyl acetate	0·03
Water/ether	0·24
Water/chloroform	4·5
Water/benzene	28
Water/light petroleum	30
0·1N HCl/trichloroethylene	100

TABLE II

DISTRIBUTION RATIOS FOR THE 1-*p*-NITROPHENYL-2-AMINO-1:3-PROPANDIOL SET FREE BY HYDROLYSIS

Buffer pH 6·0/ethyl acetate	100
Buffer pH 7·0/ethyl acetate	12
Buffer pH 8·0/ethyl acetate	2·1

At lower pH values the difference is greater. These facts have been used for estimating the amount of chloramphenicol hydrolysed in the preparations. The usually small quantities of amine are determined spectrophotometrically. E (1 per cent. 1 cm.) at 271 mμ: 397 for the hydrochloride, melting point 209–210°C. (Kofler-block). Chloramphenicol is stable enough to endure rather high acidity during the time necessary for the required separation steps[14], and it is stable on evaporating the solution and drying the residue at 100°C.

Some examples of separation procedures used in different preparations are presented in Table III.

Capsules. By using the water/ethyl acetate system the separation can be made with smaller volumes than with water/ether. Distribution ratios: dihydrostreptomycin sulphate 13; lactose > 100.

Powder for eye drops. The separation of chloramphenicol from methyl *p*-hydroxybenzoate using, for example, ethyl acetate and 0·01N sodium hydroxide, was avoided, as hydrolysis will occur. Controls were found to give low results, and a considerable amount of amine formed by hydrolysis was detected. Distribution ratios of methyl *p*-hydroxybenzoate in the system 0·1N hydrochloric acid/trichloroethylene: 0·8; boric acid in the system 0·1N hydrochloric acid/trichloroethylene: > 100 and in 0·1N hydrochloric acid/ether: 100.

Injection. Distribution ratio of dimethyl acetamide in water/ether: 54.

Ear drops. All amylocaine hydrochloride was recovered in the water phase in the system 10 ml. 0·1N hydrochloric acid/30 ml. ethyl acetate. Distribution ratio for the system buffer pH 7·0/ethyl acetate: 0·14. Loss of amylocaine base occurred on simple evaporation of the ethyl acetate solution. The distribution ratio of propylene glycol in the system water/ether: > 100.

Ointment and Suppositories. The second portion of benzene is used for "washing" purposes. The isolated substance tends to have a low melting point that cannot be avoided by using additional portions of benzene.

According to my experience it is necessary to estimate the amount of hydrolysis in chloramphenicol preparations containing water. Assays performed in order to verify the applicability of the methods to mixtures corresponding to actual preparations are reported in Table IV.

TABLE III

SEPARATION PROCEDURES FOR CHLORAMPHENICOL PREPARATIONS

Preparation	Other constituents	Separation procedure*	Comments
Capsules (2·5 g.) Chloramphenicol content *ca.* 0·75 g.	Dihydrostreptomycin sulphate Lactose	Water 25 ml./ethyl acetate 20 ml.; 3 × 3	
Powder for eye drops (0·27 g.) Chloramphenicol content *ca.* 0·050 g.	Borax Boric acid Methyl *p*-hydroxybenzoate	(1) Trichloroethylene 50 ml./0·1N HCl 5 ml; 4 × 4; Pooled HCl-phases. (2) 0·1N HCl 20 ml./ether 80 ml.; 4 × 4	
Injection (0·5 g. solution) Chloramphenicol content *ca.* 0·11 g.	Dimethyl acetamide Water	0·1N HCl 10 ml./ether 35 ml.; 3 × 2	Free amine spectrophotom. in acid phase
Ear drops (1 ml. solution) Chloramphenicol content *ca.* 0·01 g.	Propylene glycol Glycerol Water Amylocaine HCl	(1) 0·1N HCl 10 ml./ethyl acetate 30 ml.; 3 × 2; Pooled HCl-phases buffered to pH 7 and diluted to 40 ml. (2) Buffer pH 7 40 ml./ethyl acetate 50 ml.; 4 × 4	HCl: free amine + amylocaine. Ethyl acetate: Chloramphenicol. Buffer: free amine. Ethyl acetate: amylocaine.
Ointment (7 g.) Chloramphenicol content *ca.* 0·07 g.	Soft paraffin	Water 25 ml./benzene 25 ml.; 4 × 2	
Suppositories (1 g. mass) Chloramphenicol content *ca.* 0·25 g.	Cocoa butter	Water 25 ml./benzene 25 ml.; 4 × 2	

* X × Y denote extraction with X portions of the mobile phase and Y portions of the stationary phase (in Y separating funnels).

TABLE IV

RESULTS FROM VERIFICATION EXPERIMENTS ON MIXTURES CORRESPONDING TO ACTUAL PREPARATIONS

Preparation	Constituent determined	Found	Added	Properties	
				M.pt. °C.	Specific Rotation
Powder for eye drops	Chloramphenicol (gravimetric)	16·4 per cent.	16·34 per cent.	146–149	+ 18·9°
Injection	Chloramphenicol (gravimetric)	0·750 g. 0·752 g.	0·753 g. 0·754 g.	147·5–149	+ 19·4°
Ear drops	Chloramphenicol Free amine Amylocaine HCl (all spectrophotometric)	1·08 mg. 0·96 mg. 9·7 mg.	1·08 mg. 0·94 mg. 9·7 mg.		
Ointment	Chloramphenicol (spectrophotometric)	1·00 per cent. 1·00 per cent.	1·00 per cent. 1·00 per cent.	146·5–148	
	Chloramphenicol (gravimetric)	1·13 per cent.	1·12 per cent.	144–149	+ 18·0°
Suppositories	Chloramphenicol (gravimetric)	23·4 per cent.	23·1 per cent.	147·5–149·5	+ 18·7°

In the amendments of the U.S.A. Federal Register concerning chloramphenicol ointment dated September 1, 1955, the assay is a microbiological method. Two methods are given for the preparation of the sample: an emulsion technique and an extraction method.

The extraction recommended is: "Place a representative sample (0·5 gm.) in a separatory funnel containing 10 ml. of peroxide-free ether. Shake the separatory funnel vigorously to bring about complete mixing of the ointment and ether. Shake with a 15-ml. portion of 1 % phosphate buffer pH 6·0. Remove the buffer layer and repeat the extraction with two additional 15-ml. portions of buffer. Combine the extractives and dilute to 50 ml. with 1 % phosphate buffer. Make the proper estimated dilutions in 1 % phosphate buffer at pH 6·0".

From the figures in Table I it is easy to calculate that the extraction must give very low results. As the two solvents are not equilibrated with each other beforehand there will be a change in volumes. Taking this into consideration the calculated result of the extraction is 72 per cent. of the added chloramphenicol. In a practical control 73·5 per cent. was recovered in the pooled aqueous extractives.

SUMMARY

1. The possibilities of a chemical assay of choramphenicol in pharmaceutical preparations is discussed.
2. Analyses by a simple counter current technique for many different preparations are described.
3. Results from known mixtures corresponding to actual preparations verify the procedures.
4. Estimation of the degree of hydrolysis in chloramphenicol preparations containing water is recommended.
5. The extraction method for chloramphenicol in ointments recommended by the Food and Drug Administration in the U.S.A. Federal Register is criticised since it gives low results.

I wish to acknowledge my indebtedness to Professor Hans Hellberg, the Head of this Department, for advice and discussions.

REFERENCES

1. Hess, *Analyt. Chem.*, 1950, **22**, 649.
2. Garcia Madrid, *Colegio farm.*, 1952, **9**, 1.
3. Glazko, Wolf and Dill, *Arch. Biochem.*, 1949, **23**, 411.
4. Gurmendi and Ungueta, *Bol. soc. quim., Peru*, 1952, **18**, 6.
5. Truhaut, *Ann. pharm. franc.*, 1951, **9**, 347.
6. Puga, *Rev. Farm.*, 1951, **93**, 290, through *Chem. Abstr.*, 1952, **46**, 5260 h.
7. Döll, *Arzneimit.-Forsch.*, 1955, **5**, 97.
8. Bartz, *J. biol. Chem.*, 1948, **172**, 445.
9. Higuchi, Marcus and Bias, *J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 129.
10. Rebstock, Crooks, Controulis and Bartz, *J. Amer. chem. Soc.*, 1949, **71**, 2458.
11. Dony, *Une Experience Belge en Matière de Contrôle Systematique des Antibiotiques.* A report at the meeting of the Directors of Control Laboratories at the 16th General Assembly of the F.I.P. in London, 1955.
12. Controulis, Rebstock and Crooks, *J. Amer. chem. Soc.*, 1949, **71**, 2463.
13. Brunzell and Hellberg, *Ann. pharm. franç.*, 1954, **12**, 296.
14. Lennert-Petersen, *Farm. Tidende*, 1954, **64**, 66.