J. Pharm. Pharmac., 1970, **22**, 607–611 *Received January* 20, 1970

A stability study of chloramphenicol in topical formulations*

K. C. JAMES AND R. H. LEACH†

The Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cathays Park, Cardiff, and The Pharmaceutical Department, The Children's Hospital, Ladywood Road, Birmingham, U.K.

The stabilities of several chloramphenicol preparations have been investigated at ambient temperatures. The B.P.C. ointment and eye ointment were stable over 2 years. The remainder retained more than 90% of their potencies after the following periods: ear drops B.P.C., 2 years, Drug Tariff cream, 5 months and eye drops B.P.C., 3 to 4 months. 15% hydrolysis occurred after autoclaving the eye drops, and 3 to 4% after heating with a bactericide. The latter sterilization process is recommended. Two assay procedures were employed, one estimating residual chloramphenicol by ultraviolet spectrophotometry after separation from decomposition products by thin layer chromatography and the other determining the main degradation product (1-*p*-nitrophenylpropan-1,3-diol-2-amine) colorimetrically.

The chloramphenicol molecule has several active functional groups but degradation in aqueous media is almost entirely due to hydrolysis of the amide group, giving rise to 1-*p*-nitrophenylpropan-1,3-diol-2-amine (the amine) and dichloroacetic acid (Higuchi, Marcus & Bias, 1954). The reaction follows first order kinetics and although the pH of maximum stability has been reported as about 6 (Trolle-Lassen, 1953; Broadhurst & Wright, 1959) it is largely independent of pH values between 2 and 7. Degradation rates rapidly increase in alkaline media and the reaction shows both specific and general acid-base catalysis, but is independent of ionic strength (Higuchi & others, 1954).

Although several fundamental studies have been reported on the nature of chloramphenicol degradation, little work has been published on the stability of the antibiotic in formulated preparations. Haemopoietic considerations limit the systemic use of chloramphenicol but it is still one of the most widely used antibiotics for local application. Several pharmacopoeias and formularies include details of topical dosage forms but frequently exclude an indication of shelf life. This paper describes stability studies on the topical formulations included in British reference compendia.

EXPERIMENTAL

Analytical methods

Amine method. This method was developed by Brunzell (1957) and involves the coupling of the amine produced on degradation of chloramphenicol with 1,2-naphthaquinone-4-sodium sulphonate and estimating the resulting colour photometrically.

* This work forms part of a thesis by R. H. Leach for the degree of M.Pharm. of the University of Wales.

† Present address: Computer Services Unit, The Queen Elizabeth Hospital, Edgbaston, Birmingham, 15, and the University of Aston in Birmingham, U.K.

The residual antibiotic in degraded chloramphenicol mixtures can be calculated by subtracting the chloramphenicol molar equivalent of the amine from the original content.

In a preliminary experiment when 10 replicate samples of amine were assayed, the variation between the extreme figures was 2·7% and the maximum deviation from the mean 1·6%. This reproducibility was considered satisfactory, since its effect on figures for residual chloramphenicol is small, thus for example for a sample which is 10% degraded, the error in the residual chloramphenicol would be a tenth of that of the amine.

Experiments showed that neither the eye or ear drop vehicles nor the cream base (49% hard macrogol B.P.C. and 50% propylene glycol) interfered with the estimation.

Thin-layer chromatographic method. Since the amine method was not applicable to all samples, and under-estimated decomposition in heavily degraded samples, where secondary reactions become significant, a second method was used. This estimated residual chloramphenicol by spectrophotometry after a thin-layer chromatographic separation from decomposition products which also absorb in the ultraviolet region.

Schwarn, Dabner & others (1966) reported a thin-layer chromatographic separation of chloramphenicol from degradation products on Silica gel HF 254 using chloroform–isopropanol (80 : 20) as ascending phase. The spot was located under ultraviolet light at 254 nm, removed quantitatively and extracted with ethanol for spectrophotometric determination. Good agreement was reported between results from this assay and those obtained from two microbiological methods. Investigation of the technique showed that chloramphenicol, the amine, *p*-nitrobenzaldehyde and *p*-nitrobenzoic acid could be separated. The last two derivatives have been suggested, together with several condensation products, as intermediates in severely degraded chloramphenicol solutions (Knabe & Kraeuter, 1963; Lacharme & Netien, 1964).

Whenever possible, determinations using both analytical procedures were made in the shelf life studies and periodically results were compared with the cup plate assay.

Application of formulated preparations for thin-layer separation

The ophthalmic solution could be applied directly to the plate, as were the ear drops, after weight sampling and dilution. When the cream was spotted and run in the usual way a diffuse dumb-bell shaped spot formed near the origin and no partition occurred. Modification of the ascending phase failed to give adequate separation, so the cream was examined using Brunzell's method only. The ointment (1% in soft paraffin) and the eye ointment (1% in soft paraffin containing 10% wool fat) were dispersed in chloroform for application to the plate. The interference caused by the greasy bases was overcome by running the plate in two solvents. An initial run in ether took the vehicle to the top of the plate, while a second run in the usual solvent, until the front was just below the greasy layer, separated the chloramphenicol as before.

Quantitative removal of the spot was effected by scraping off the absorbent with a vulcanite spatula in a draught free environment into a funnel in a test-tube. Any adherent powder was first tapped then washed into the tube with ethanol. After 4 h, or overnight, when extraction was complete, the ethanolic solution was centrifuged for 5 min at 2000 rev/min and the supernatant estimated spectrophotometrically (E, 1%, 1 cm chloramphenicol in ethanol at 274 nm = 308). Schwarn's description of the process (1966) contains few practical details. Those we used together with the reproducibility of the results, are given in Table 1.

Table 1. *Thin-layer chromatographic assay of chloramphenicol preparations with recoveries and practical details*

Preparation	Pre-treatment of sample	Volume spotted (μl)	Volume of ethanol for extraction (ml)	Recovery %	Coefficient of variation: Single assay	Coefficient of variation: Mean of 4 assays
Eye drops (0·5%)	None	25	10	97·1	3·3	1·7
Ear drops (5%)	1 g in water adjusted to 20 ml	25	5	97·1	3·3	1·7
Eye ointment (1%)	0·70 g in chloroform adjusted to 10 ml	50	5	97·5	8·1	4·1
Ointment	0·70 g in chloroform adjusted to 10 ml	50	5	97·4	9·5	4·8

During the stability investigations all assays were made in quadruplicate. Each time a sample was submitted for assay, fresh standard solutions of chloramphenicol were also run in quadruplicate and the percentage recovery calculated. The mean recovery of standards during the investigation was 97·6% and the extreme values were 95·3 and 101%. The recoveries of the unknown were assumed to equal those of the standards.

Stability investigation

Chloramphenicol ear drops B.P.C. A sample was prepared, assayed by direct spectrophotometry, packed in a dry actinic stoppered bottle and stored at 20–25° for about 2 years. Samples were withdrawn at intervals and assayed using the procedures described above. Results are in Table 2.

Table 2. *The stability of some chloramphenicol topical formulations*

	Chloramphenicol remaining %							
	Ear drops		Cream (Drug Tariff) formula	Eye drops: Refrigeration 0–4°		Eye drops: Room temperature 20–25°		
Storage time (weeks)	Amine method (99·7)*	TLC method	Amine method (110)*	Amine method (99·6)*	TLC method	Amine method	(99·6)*	TLC method
0	100	99·6	100	100	100	100		100
0	—	—	—	97·2†	98·1†	97·2†		98·1†
4	—	—	98·6	—	—	—		—
8	98·5	96·7	96·6	96·9	95·6	94·3		94·3
16	98·0	94·7	94·2	96·6	98·1	91·5		90·5
20	—	—	93·0	—	—	—		—
24	97·7	96·1	91·2	96·5	93·5	83·3	(92·4)‡	88·3
32	—	—	90·2	—	—	—		—
40	96·9	95·7	87·5	95·9	95·0	81·6		81·1
48	—	—	85·0	—	—	—		—
52	95·9	97·4	84·0	96·1	94·4	77·9	(77·8)‡	75·7
76	94·5	94·5	—	95·0	93·2	71·6		67·2
88	94·9	94·6	—	94·5	94·9	69·0		67·0
96	96·5	93·7	—	94·6	97·3	64·0		68·9

*Initial assay by direct spectrophotometry.
†After sterilization.
‡Cup plate assay.

Chloramphenicol cream. Drug Tariff Formula. After its preparation and assay, the sample was stored in a screw capped actinic glass jar at 20–25° C. The results of assays at various intervals are in Table 2. The marked decrease in stability of the cream compared with the ear drops is surprising, since the only difference in formulae is the replacement of 49% of propylene glycol by hard macrogol B.P.C., to solidify the preparation. It was established, by subjecting carefully dried raw materials to suitable challenges, followed by factorial analysis, that the increased degradation was due to moisture, and that the macrogol had no effect on the reaction. A sample of hard macrogol B.P.C. used in the cream gave a figure of 1·2% water compared with 0·21% for the propylene glycol used in the eardrops. These results together with the known hydrophilic nature of the macrogols support the conclusions.

Chloramphenicol eye ointment and ointment B.P.C. Samples of the eye ointment, prepared aseptically, and the ointment were assayed both by the B.P.C. extraction method and the TLC method described previously. The ointments were packed in screw capped actinic glass jars, from which aliquots were withdrawn at intervals for TLC analysis. The results obtained gave no evidence of degradation within the limits of reproducibility of the assay. Confirmation of this was given by qualitative examination of the thin-layer plates, which showed no new spots over the two year study period.

Chloramphenicol eye drops B.P.C. A preliminary study was made using Brunzell's method to assess the possibility of heat sterilization of these drops, which are currently processed by filtration. Sterilization by heating with a bactericide (the phenylmercuric nitrate present in the formulation) at 100° for 30 min resulted in 3–4% hydrolysis, while autoclaving at 115° for the same period resulted in about 15% degradation. In neither case was any degradation product other than the amine seen on the thin-layer chromatogram. Heat treatment was followed by rapid cooling in both cases. The results of a stability investigation at two temperatures on a sample of eye drops packed in ampoules and sterilized by heating with a bactericide are shown in Table 2.

DISCUSSION

Shelf lives at 20 to 25°, can be inferred from the above results. The ointment and the eye ointment can be expected to retain their potencies for over two years. The ear drops and the cream, provided precautions are taken to exclude moisture can be given anticipated shelf lives of two years with less than 10% overage. However, since it is difficult to completely exclude moisture from the cream, 5 months is probably a more realistic estimate. A shelf life of two years can be predicted for the eye drops with 10% overage if stored at 0 to 4°, but only 3 to 4 months can be recommended at 20 to 25°.

The eye drops study may rationalize the very different approaches adopted for chloramphenicol ophthalmic solutions in various pharmacopoeias and formularies. For example, the United States Pharmacopeia 17 prescribes reconstitution of freeze dried buffered powder, the British Pharmaceutical Codex 1968 and the Australian Pharmaceutical Formulary 1964 recommend sterilization by filtration, while the Pharmacopoeia Nordica 1963 sterilizes the drops at 100° for 20 min. The results confirm the suitability of heat sterilization with a bactericide at 100° followed by rapid cooling. Since such solutions retain potency for considerable periods, particularly on

refrigeration, perhaps the British Pharmaceutical Codex should give consideration to this alternative method of manufacture.

The estimates of shelf life data presented above would only be valid if the amine formed on decomposition were non-toxic. The possibility of local or systemic toxicity seems little more than a theoretical hazard since the amine is a normal metabolite of chloramphenicol (Glazko, Dill & Rebstock, 1950). Further, at the Birmingham and Midland Eye Hospital, all chloramphenicol eye drops have been autoclaved for some 3 years, and are still considered to be one of the blandest eye preparations available (Roper-Hall: personal communication). Similarly, intrathecal chloramphenicol, although rarely used these days is frequently sterilized by autoclaving, apparently without the development of adverse reactions.

Acknowledgements

Thanks are due to Messrs. Parke Davis & Company Ltd., for kindly making the microbiological assays and to Birmingham Children's Hospital for facilities provided.

REFERENCES

BROADHURST, N. & WRIGHT, S. E. (1959). *Aust. J. Pharm.*, 106–107.

BRUNZELL, A. (1957). *Svensk. farm. Tidskr.*, **6**, 129–138.

GLAZKO, A. J., DILL, W. A. & REBSTOCK, M. C. (1950). *J. biol. Chem.*, **183**, 679–691.

HIGUCHI, T., MARCUS, A. D. & BIAS, C. D. (1954). *J. Am. Pharm. Ass. (Sci. Edn)*, **43**, 129–134.

HIGUCHI, T. & MARCUS, A. D. (1954). *Ibid.*, **43**, 530–535.

KNABE, J. & KRAEUTER, R. (1963). *Arch. Pharm., Berl.*, **296**, 190–205.

LACHARME, J. & NETIEN, G. (1964). *Bull. Trav. Soc. Pharm. Lyon*, **3**, 122–128.

SCHWARN, E. F., DABNER, C., WILSON, J. W., BOGHOSIAN, M. P. (1966). *J. pharm. Sci.*, **55**, 744–745.

TROLLE-LASSEN, C. (1953). *Arch. Pharm. Chemi*, **60**, 689.