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A Simple Qualitative Test for Deterioration of Cycloserine *

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An accompanying paper from this Centre[c] reports deterioration of cycloserine in humid tropical climates and recommends various preventive measures. However, in spite of all precautions, it would be advisable for those using cycloserine in humid climates to ensure periodically that there. is no loss of drug content. Quantitative assay procedures (for example, that described by Jones [d]), although very desirable, are cumbersome and cannot be undertaken in institutions with limited laboratory facilities. A simple qualitative test that can be used under these circumstances is described below. The test is based on the observation that samples of deteriorated cycloserine form yellow solutions when dissolved in dilute alkali.

Materials and methods

The stated content of the tablets and capsules of cycloserine used in the present investigation was 250 mg; our findings, however, indicate that the content was frequently about 5% higher. It is stated in the *British Pharmacopoeia* [e, f] that, taking into account tolerances for manufacturing variations, 92.5%–107.5% of the stated content are acceptable limits for tablets, and 90.0%–110.0% are acceptable limits for capsules (B.P. limits).

The qualitative test has been designed to detect deterioration if the cycloserine content of tablets or capsules falls below 225 mg, that is, below 90% of the stated content of 250 mg. In view of the excess content of approximately 5%, this implies that the test is designed to detect deterioration of about 15 % or more.

Qualitative test. Each tablet was ground up with 5 ml of 0.4 N sodium hydroxide solution in a glass mortar and the resulting suspension was transferred to a test-tube. The contents of each capsule were directly added to the alkali in a test-tube and mixed well.

The presence of a yellow colour in the suspension within 5 minutes was regarded as a " positive " finding. Suspensions which were light yellow or white were classified as "negative." (To facilitate standardization in reading the colour reaction, a suspension of 150 mg of calcium carbonate in 5 ml of 0.01 % potassium dichromate solution may be employed as a standard positive.)

Quantitative assay. After the completion of the qualitative test, the cycloserine content of the solution in the test-tube plus any residual material on the mortar or pestle or both was determined by the method of Jones,[d] using pure cycloserine as a standard. Pure cycloserine was supplied by the manufacturers in powder form in heat-sealed, amber-coloured ampoules, and stored at the Centre in a cold-room at a temperature of approximately 9°C.

Results of Investigation [1]

A preliminary investigation was undertaken to compare the findings of the qualitative test with 2 different concentrations of sodium hydroxide solution (0.4 N and 0.5 N). For this purpose, a total of 57 tablets and 38 capsules were taken from stocks that were known to be fully potent (that is, each contained at least 250 mg cycloserine), and from stocks that were known to have deteriorated. The findings are presented in Table 1.

It can be seen that a positive reaction with 0.4 N alkali indicates always that the cycloserine content, whether of tablets or capsules, is less than 90% of the stated content. In contrast, with 0.5 N alkali, a positive reaction was obtained even when

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[c] Rao, K.V.N., Eidus, L., Evans, C., Kailasam, S., Radhakrishna, S., Somasundaram, P.R., Stott, H., Subbammal, S. & Tripathy, S. P. (1968) *Bull. Wld Hlth* Org., **39,** 781.

[d] Jones, L. R. (1956) *Analyt. Chem.,* **28,** 39.

[e] *British Pharmacopoeia,* 1963, London, Pharmaceutical Press, p. 221.

[f] *British Pharmacopoeia, Addendum*. 1966, London Pharmaceutical Press, p. 23.

TABLE 1

RESULTS OF QUALITATIVE TESTS WITH 0.4 N AND 0.5 N SODIUM HYDROXIDE, RELATED TO CYCLOSERINE CONTENT

Sodium hydroxide concentration	Form of cycloserine	Positive result		Negative result	
		No. of specimens	Range of cycloserine content (%) [a]	No. of specimens	Range of cycloserine content (%) [a]
0.4 N	Tablets	22	70-86	9	91-106
	Capsules	9	62-86	14	87-106
0.5 N	Tablets	11	65-96	15	98-104
	Capsules	7	91-99	8	99-106

[a] Expressed as a percentage of the stated content of 250 mg.

the cycloserine content was as high as 96% for tablets and 99% for capsules. As the aim of the test is to detect a fall in content to less than 90% of the stated amount, the findings with 0.4 N alkali are clearly more satisfactory. Consequently, 0.4 N alkali alone was used in the second investigation.

Results of Investigation 2

Since cycloserine is normally dispensed in *tablet* form at this Centre, the relationship between the results of the qualitative and quantitative tests was studied in a further batch of 62 tablets, 10 of which were known to be fully potent while the remainder was from batches known to contain at least 70% of the stated cycloserine content. The tablets were sent under code numbers to the biochemist, who examined them by the qualitative test ; next, the test-tubes containing the cycloserine solutions were given new code numbers and quantitative assays were undertaken. The findings are presented in Table 2.

Of the 26 tablets which yielded a positive result, 23 (88%) had a cycloserine content of less than 90%, while the remaining 3 had a content of 91% each. In contrast, only 6 (17%) of the 36 tablets showing a negative result hod a content of less than 90%, the lowest value being 83 % ; of the remaining 30, 21 had a content of at least 93 %.

In summary, these investigations have shown that a positive result in the qualitative test always indicates that the drug content is less than the minimum B.P. limit, and a negative result indicates that the drug content is 80% or more of the stated content.

Agreement between duplicate observers in reading the test reaction

Of the 62 tests in Investigation 2, 41 were read independently by a second reader, who had no previous experience with the test. Both readers classified 10 reactions as positive (80%-91 % of stated content of cycloserine) and 30 as negative (83%-106 % of the stated content). One test reaction was classified as negative by the experienced reader and as positive by the other; the cycloserine content of this tablet was 91%.

Comments

We have observed that deteriorated tablets and capsules of cycloserine, when dissolved in dilute

TABLE 2

RESULTS OF QUALITATIVE AND QUANTITATIVE TESTS ON CYCLOSERINE TABLETS

Cycloserine content (%) [a]	Qualitative test result [b]	
	Positive	Negative
< 80	4	0
80-84	10	1
85-89	9	5
90-92	3	9
93-94	0	9
> 95	0	12
Total number of tablets	26	36
Range of cycloserine content (%) [a]	70-91	83-106

[a] Expressed as a percentage of the stated content of 250 mg.
[b] Using 0.4 N sodium hydroxide solution.

alkali, yield yellow solutions which strongly absorb ultraviolet light in the 280 nm–290 nm band and give a positive biuret reaction. This indicates that the deteriorated product is of a diketopiperazine nature, an observation which is in agreement with that of Hidy et al.[8] that cycloserine dimerizes in solution into 2,5-bis-(aminoxymethyl) 3,6-diketopiperazine.

The biuret reaction is very sensitive, that is, the result is positive even if the extent of deterioration is very small. It is therefore not suitable, particularly since the cycloserine content of tablets or capsules is frequently about 5% more than that stated, for screening batches of cycloserine as satisfactory or unsatisfactory, employing B.P. limits.

It has been demonstrated that, *if the initial cycloserine content is about 5% in excess of the stated amount,* a positive result with the qualitative test *always* indicates that the cycloserine content is less than the minimum B.P. limit. In practice, therefore, a useful working rule would be to reject all batches with a positive result as unsatisfactory. Although such a rule will usually ensure that *only* unsatisfactory batches are rejected, it cannot ensure that all batches which are accepted as satisfactory are, in fact, above the minimum B.P. limit. For instance, in the present study, 1 of 11 tablets with a content of 80%–84%, 5 of 14 with a content of 85%–89% and 9 of 12 with a content of 90%–92% of the stated content yielded a negative result, although the content was less than the minimum B.P. limit.

[8] Hidy, P. H. et al. (1955) *J. Amer. chem. Soc.,* **77,** 2345.

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