COLORIMETRIC ESTIMATION OF DIHYDROSTREPTOMYCIN AND STREPTOMYCIN

By R. Natarajan and J. N. Tayal

T.D.E. Laboratories, Kanpur, India

Received November 30, 1956

The pharmacopoeias have recommended microbiological methods for estimating the potency of streptomycin and dihydrostreptomycin. A number of chemical methods have been described in literature[1–7], and of these, one estimates both streptomycin and dihydrostreptomycin in a combined form[2]. It consists of measuring the red colour produced by a mixture of these antibiotics with α-naphthol and sodium hypobromite in an alkaline medium under ice-cold conditions. We observed that (i) it is not possible to measure optical density at such a low temperature due to condensation of moisture on the sides of the cell; (ii) due to addition of urea, nitrogen gas is formed which interferes with the quick and accurate measurement of the optical density; the colour produced is not stable and (iii) the limit of concentration up to which Beer's law is followed is too low (0·4 mg. of the substance). With the help of a modified method, which is quick and reliable, estimates of samples of dihydrostreptomycin have been found to compare favourably with the figures obtained by the standard microbiological method of assay described in the British Pharmacopoeia 1953.

Experimental

Reagents. 10 per cent NaOH solution, 0·02 per cent α-naphthol solution in absolute ethanol, carbon tetrachloride (B.P.), absolute ethanol (B.P.), and sodium hypobromite.

The sodium hypobromite reagent was prepared by dissolving 20 g. of NaOH in 75 ml. of distilled water, cooling and adding 5 ml. of bromine; after solution, distilled water was added to 100 ml., 10 ml. of this was diluted to 100 ml. with distilled water.

Preliminary experiments. To remove the difficulties encountered in the procedure recommended by Buch and colleagues[2], excess bromine was removed by organic solvents. Ten ml. of carbon tetrachloride for each reaction helps to stabilise the colour; but the absorption values obtained with the aqueous layer, though found to observe Beer's Law, were slightly low, indicating that the reaction product might be partially retained by the carbon tetrachloride. Also the aqueous layer was slightly turbid, but this could be clarified with an equal volume of absolute ethanol which also restored the colour to the carbon tetrachloride layer.

Minimum percentage transmission. The minimum percentage transmission was obtained at 530 mμ and was found to remain stable for about 10 minutes after the addition of NaOBr reagent. Percentage transmissions of blank, three concentrations of the standard and one sample can be measured well within this period.

Procedure. Solutions of dihydrostreptomycin or streptomycin (known standards) were made in distilled water, the strengths of the different solutions varied from 0·25 to 2·5 mg./ml. One ml. of each solution was taken in a 50 ml. stoppered cylinder; 1 ml. of 10 per cent NaOH and 2 ml. of α-naphthol were added to each cylinder. The contents of the cylinder were shaken and allowed to stand for 15 minutes; 1 ml. of NaOBr reagent was then added and within half a minute 10 ml. of carbon tetrachloride was added; the contents were again shaken well; 5 ml. of absolute ethanol was subsequently added to each cylinder and the contents shaken well. The liquids were allowed to separate; without disturbing the layers, a portion of the aqueous layer from each was pipetted into 1 cm. cells and the percentage transmission measured by using a blank (prepared in the same way as the standard with 1 ml. of distilled water in place of the sample) kept at 100 per cent transmission at 530 mμ. The entire procedure was carried out at room temperature (30°).

This procedure was employed for estimating the potency of samples of dihydrostreptomycin by intrapolating the percentage transmission values of the samples with a standard curve drawn from values obtained with known strengths of a standard preparation. The microbiological method of assay employed for estimating the potency of the samples is that of the British Pharmacopoeia 1953.

Results and Discussion

Beer's Law. Percentage transmission of 0·5, 1·0, 1·5, 2·0 and 2·5 mg./ml. of dihydrostreptomycin was measured using the same reagents. Values obtained are given in Table I. Six estimations were made for each concentration. Similarly, 0·5, 0·75, 1·0, 1·25 and 1·5 mg./ml. of streptomycin sulphate was taken and the values of percentage transmission in relation to these are also recorded in Table I. The reaction was found to follow Beer's Law up to a concentration of 1·5 mg./ml. of dihydrostreptomycin and streptomycin sulphate.

TABLE I

PERCENTAGE TRANSMISSION OF THE TWO ANTIBIOTICS IN MG./ML. CONCENTRATIONS (AVERAGE OF SIX READINGS)

Dihydrostreptomycin sulphate mg.	Per cent transmission	Streptomycin sulphate mg.	Per cent transmission
0·5	92·5	0·5	94·0
1·0	84·8	0·75	91·05
1·5	77·5	1·0	88·0
2·0	71·25	1·25	85·1
2·5	65·5	1·5	82·15

After establishing the range of concentration within which the reaction follows Beer's Law, values of percentage transmission were obtained with 0·25, 0·50, 0·75, 1·0, 1·25 and 1·50 mg./ml. of dihydrostreptomycin sulphate. Three estimations were made for each concentration using different sets of reagents on different days. Although the values of percentage transmission obtained on different days were found to follow Beer's Law

independently, they tended to differ slightly among themselves. It is, therefore, advisable to draw a fresh standard curve, whenever this method is to be employed for the estimation of dihydrostreptomycin or streptomycin sulphate.

Comparison of Proposed Colorimetric Method with the B.P. Bioassay Method

To assess the suitability of the colorimetric method for the estimation of the potency of samples of dihydrostreptomycin, six samples of dihydrostreptomycin sulphate manufactured by a reputable firm were selected; these samples were found to conform to the B.P. test for "limit of streptomycin".

TABLE II

COMPARATIVE FIGURES OF DIHYDROSTREPTOMYCIN CONTENT BY THE COLORIMETRIC METHOD AND THE B.P. MICROBIOLOGICAL METHOD

Sample No.	Dihydrostreptomycin sulphate content units/mg.		Deviation from the microbiological method, per cent
	By the suggested method	By B.P. microbiological method	
1	658	675	−2·5
2	665	663	+0·3
3	675	673	+0·3
4	658	670	−1·8
5	658	661	−0·45
6	658	681	−3·4

The potencies of these samples were estimated by intrapolating the percentage transmission values, obtained with a known concentration of the sample with a standard curve drawn with the percentage transmission values plotted against known potency of the standard in terms of units/mg. The same samples were assayed by the microbiological method of assay and the results obtained by the colorimetric method compared with those obtained by the microbiological method. These results are tabulated in Table II. From this, it will be seen that there is good agreement between the values obtained by the proposed colorimetric and the standard microbiological methods; the deviations in values between the two methods fall within a narrow range of experimental error.

SUMMARY

1. A colorimetric method for the estimation of dihydrostreptomycin and streptomycin sulphate has been described.

2. The values obtained compare favourably with those of the B.P. microbiological assay.

3. As the colour reaction is equally applicable both to streptomycin and dihydrostreptomycin it has been recommended that in the case of samples of dihydrostreptomycin sulphate, the proposed colorimetric method should be used in conjunction with the B.P. test standard for the limit of streptomycin in dihydrostreptomycin.

Guidance during the course of this investigation from Dr. T. S. Subramanian is gratefully acknowledged.

REFERENCES

1. Ashton, Foster and Fatherley, *Analyst*, 1953, **78**, 581.
2. Buch, Mader and Fredioni, *Bol. Col. Quim*, 1949, puetro Rico 6, 6–7, 9; through *Chem. Abstr.*, 1950, 281 p.
3. Halliday, *Nature, Lond.*, 1952, **169**, 335.
4. Monastero, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 322.
5. Page, *J. Pharm. Pharmacol.*, 1952, **4**, 13.
6. Szafir and Bennett, *Science*, 1953, **117**, 717.
7. Vail and Bricker, *Analyt. Chem.*, 1952, **24**, 975.