A NOTE ON THE COLORIMETRIC ASSAY OF CORTISONE AND HYDROCORTISONE

BY (MISS) SULTANA ANSARI AND RIAZ AHMED KHAN

From the Glaxo Laboratories (Pakistan) Ltd., Karachi

Received November 19, 1959

A colorimetric method of estimation of cortisone and hydrocortisone in pharmaceutical preparations using 2,6-di-t-butyl-*p*-cresol has been simplified in its details of procedure and compared with the tetrazolium method. Preparations of cortisone and hydrocortisone containing penicillin, streptomycin and neomycin have also been examined.

SCHULZ and Neuss[1] have proposed a method for the estimation of corticosteroids which is based on the interaction of cyclic ketones with phenols[2] to form coloured complexes. In this method the two steroids, cortisone and hydrocortisone react with an alkaline solution of 2,6-di-t-butyl-*p*-cresol to form yellow-brown and blue colours respectively. A comparison of the absorbance of the sample is made with the colour obtained by an approximate concentration of the standard. We have endeavoured to simplify and standardise the procedure so that a standard curve for definite concentrations can be obtained. The modified method has been compared with the tetrazolium reagent method[3] of the United States Pharmacopeia[4].

The pharmaceutical preparations studied were cortisone and hydrocortisone skin and eye ointments, lotions, injections and tablets and also combined preparations of the steroids with antibiotics.

EXPERIMENTAL

Apparatus. Unicam Spectrophotometer, SP600; reflux condenser, ground-glass joints; round bottomed flasks 100 ml. with ground glass joints; steam bath, capable of being maintained at 100°.

Reagents. Ethanol 90 per cent w/v; DTBPC reagent: 2,6-di-t-butyl-*p*-cresol 0·5 per cent, solution in 90 per cent ethanol; N sodium hydroxide freshly prepared and standardized; strong solution of the standard corticosteroid in ethanol (0·5 mg./ml.).

Procedure

Standard curve. Dilute 0·25, 0·5, 0·75, 1·0, 1·25 and 1·5 ml. of the strong standard solution of the steroid to 25 ml. with ethanol separately in volumetric flasks. Pipette 5·0 ml. of each solution into a 100 ml. round bottomed flask, add 5·0 ml. of the DTBPC reagent followed by 10 ml. of the sodium hydroxide solution. Reflux for 30 minutes in a steam bath maintained at 100°. (A boiling water bath with a stirrer to maintain the temperature at 100° can also be used). Without removing the condenser, immerse the flask for two minutes in cold water at 10 to 12°, rotating it gently. Remove the flask from the water and allow it to stand at room

temperature for 10 minutes. Filter rapidly through a filter paper (Whatman No. 41) avoiding loss of ethanol due to evaporation. Determine the absorbance of the filtrate at 625 mμ for hydrocortisone and 471 mμ for cortisone, in a 1 cm. cell with distilled water as blank. The time interval between the reading and removing the flask from the steam bath should be between 20 and 22 minutes. Carry out a blank determination on 5·0 ml. of ethanol and the reagents in the same manner and subtract the blank from the above readings. The curve is plotted with concentration and absorbance as abscissa and ordinate respectively. Standard curves for cortisone and hydrocortisone are shown in Figures 1 and 2.

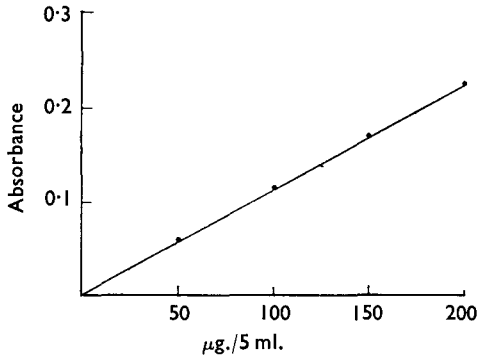

FIG. 1. Standard curve for hydrocortisone.

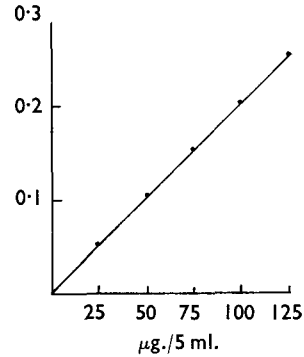

FIG. 2. Standard curve for cortisone acetate.

Ointments, lotions and injections. Weigh or pipette a sufficient quantity of the sample to contain about 10 mg. of the steroid in a 100 ml. volumetric flask. Extract the steroid with ethanol by warming on a water bath. Cool and make up to volume. Filter through sintered glass (porosity, 15 to 40 μ). Pipette 10 ml. of the filtrate and make up to 50 ml. in a volumetric flask. Pipette 5 ml. of the final dilution (equivalent to 100 μg.) into a 100 ml. round bottomed stoppered flask and proceed as described under the method for the standard curve. Correct the absorbance by treating the reagents in the same way and using 5 ml. of ethanol in place of the sample. (With ointments having a greasy base, make a blank determination on the paraffins used in the preparation of the ointment).

Tablets. Weigh sufficient quantity of powdered tablets to contain 10 mg. of the steroid into a volumetric flask. Extract the steroid as above with 100 ml. of chloroform. Filter, taking care to avoid loss of chloroform by evaporation. Prepare suitable sub-dilutions as described above, so that 5·0 ml. contains about 100 μg. of the steroid. Evaporate 5·0 ml. of the chloroform extract to dryness in a 100 ml. round-bottomed stoppered flask and dissolve the residue in 5·0 ml. of ethanol. Develop and read the colour as in the procedure described above.

Recovery. Weigh a definite quantity of the standard steroid and mix thoroughly with the pharmaceutical preparation (in the case of the tablets,

in the powdered granules). Proceed as described and calculate the quantity of the steroid found in the sample from the total quantity determined by the procedure and report as per cent recovery of the standard steroid.

Comparison with Tetrazolium Method. Proceed as described in the United States Pharmacopeia[4] for the determination of the steroid in the sample. The results are summarised in Table I.

TABLE I

Steroid	Sample	Age	Claimed per cent	Tetrazolium method	DTBPC method	
				Found per cent	Found per cent	Per cent recovery of added steroid
Cortisone (acetate)	Cortisone eye ointment (greasy base)	6 months	1·0	0·98	1·0	100·1
,,	Cortisone tablets	3 months	8·3	7·7	7·9	97·3
Hydrocortisone (alcohol)	Hydrocortisone skin ointment	5 months	1·0	0·98	1·04	101·8
,,	Hydrocortisone skin lotion	12 months	0·50	0·51	0·55	101·5
Hydrocortisone (hemisuccinate sodium)	Intravenous hydrocortisone	17 months	74·6		65·3	101·8
Hydrocortisone (acetate)	Hydrocortisone with neomycin skin ointment	30 months	1·0		0·93	100·5
,,	Hydrocortisone with penicillin and dihydrostreptomycin ointment (greasy base)	2 months	0·50		0·49	100·5

Discussion

We found that magnetic stirring was not necessary and we preferred a constant boiling steam bath. The cooling period of ten minutes in the original method[1] was found to be unnecessarily long and rapid cooling for two minutes followed by standing for 10 minutes at room temperature stabilised the colour for another 15 minutes and the reading was made after a total time of 20 minutes from the removal of the flask from the bath.

The use of 5 per cent aqueous sodium hydroxide solution as suggested by Schulz and Neuss gave readings which were not reproducible and did not show a linear relationship. To eliminate the error due to the quantitative difference in the alkali strength in the determinations, it was decided to use freshly standardised N sodium hydroxide solution, as a result of which reproducible readings for a calibration curve were obtained.

The strength of DTBPC reagent was reduced to 0·5 per cent and this did not effect the accuracy of results. The ethanol was standardised at 90 per cent as 95 per cent strength gave turbidity to the reaction mixture on dilution. Also further dilution to 25 ml. after filtration or addition of 5·0 ml. of ethanol to make up the volume as directed in the original method was found to introduce errors. With these modifications, we found that

with cortisone, Beer's Law was obeyed up to 125 μg./5 ml., and with hydrocortisone, the highest concentration was 200 μg./5 ml.

Reagent blanks in different determinations ranged from 0·003 to 0·006 at 625 mμ and from 0·028 to 0·032 at 471 mμ.

The DTBPC method of estimation of cortisone and hydrocortisone offers an advantage over the official tetrazolium method because of simplicity of operation as well as the development of different colours for the two steroids. We found that the tetrazolium method gave low results in our estimations, while the results by the DTBPC method were slightly high. Antibiotics like penicillin, streptomycin and neomycin did not interfere in the determination of the steroids in the preparations examined by us. The calibration curve holds good to ± 2 per cent for routine determinations and needs to be checked frequently. For very accurate determinations, we found that there was no alternative to taking comparative readings of samples and standards of near concentrations and determining the steroid content by ratio.

Acknowledgements. We acknowledge the helpful suggestions made by Mr. D. C. M. Adamson, Chief Analyst, Glaxo Laboratories Ltd., Greenford, during the preparation of this paper.

References

1. Schulz and Neuss, *Analyt. Chem.*, 1957, **29**, 1662.
2. Dane and Schmitt, *Annalen*, 1938, **536**, 198.
3. Mader and Buck, *Analyt. Chem.*, 1952, **24**, 666.
4. *United States Pharmacopeia*, 1955, XV Revision, 180.