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Colorimetric method for the estimation of norethynodrel in tablets containing mestranol

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The colour method proposed for the estimation of norethynodrel in tablets with mestranol utilises a reaction similar to the well known Zimmerman reaction, the reagents employed being *m*-dinitrobenzene and benzyltrimethylammonium hydroxide solution and a solvent mixture consisting of ethyl acetate and ethanol. Maximum extinction was at a wavelength of 510 mμ and Beer's law is obeyed for a concentration range 5 to 20μg norethynodrel per ml final solution.

AN attempt to use a conventional method for estimating norethynodrel utilising light absorption was unsatisfactory since the material exhibited little characteristic absorption, whereas mestranol had a significant absorption at a wavelength at about 280 mμ.

The first procedure adopted in this Laboratory was an extension of the British Pharmacopoeia method for methyltestosterone tablets involving the precipitation of the 3-ketosteroid with an acid solution of 2,4-dinitrophenylhydrazine. The precipitate was collected by filtration and ultimately for light extinction of a chloroform solution of the derivative which was compared against that obtained by similarly treating a known standard solution of norethynodrel. Maximum extinction was at 390 mμ and Beer's law was obeyed over the range 2·5 to 10 μg norethynodrel per ml expressed as final concentration of solution.

Although satisfactory results were obtained by this method it involved several lengthy manipulations and suffered from the disadvantage that complete precipitation of the 2,4-dinitrophenylhydrazone occurred only on prolonged standing. A quicker method was therefore sought.

Experimental

REAGENTS

Analar *m*-dinitrobenzene, used to prepare a 0·5% w/v solution in ethanol: benzyltrimethylammonium hydroxide (40% w/w aqueous solution) and used at this concentration (Corker, Norymberski & Throw, 1962): Analar ethyl acetate. Norethynodrel (Roussel-UCLAF).

PREPARATION OF CALIBRATION CURVE

The optimum intensity of colour consistent with simplicity of manipulation and reproducible results was achieved as follows:

A chloroform solution of norethynodrel (0·005%) was prepared and quantities equivalent to 50, 100, 150 and 200 μg transferred to separate 10 ml graduated flasks and the contents evaporated to dryness in a boiling water-bath. A fifth flask was used to prepare a reagent blank.

The solid residue in each flask was dissolved by the addition of 0·3 ml of a solution of *m*-dinitrobenzene, followed with mixing by 0·15 ml of benzyltrimethylammonium hydroxide solution.

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After 30 min at 20°±0·5° the contents of the flasks were diluted to volume with 10% v/v ethanol in ethyl acetate.

The extinctions of the red-violet colour were read immediately on a spectrophotometer (Uvispec) in 1 cm cells at 510 mμ and the calibration curve plotted from the results. This was a straight line which passed through the origin and Beer's law was obeyed over the range of concentrations employed.

ESTIMATION OF NORETHYNODREL IN TABLETS

Several tablets were reduced to a fine powder and an amount of powder equivalent to about 2·5 mg norethynodrel accurately weighed and transferred to a 100 ml graduated flask. Approximately 70 ml of chloroform was added, the mixture shaken for 15 min and the volume made up to 100 ml at 20° with chloroform.

At the same time a standard chloroform solution of norethynodrel (0·0025%) was prepared. Quantities of norethynodrel equivalent to 125 μg (= 5 ml) were transferred to separate 10 ml graduated flasks from the standard and text solutions. A third 10 ml graduated flask containing 5 ml of chloroform was used to prepare a reagent blank. The content of each flask was evaporated to dryness in a boiling water-bath and the assay completed as described for the calibration.

The extinction of the test and standard colours was compared against the reagent blank.

Discussion

The reaction between norethynodrel and *m*-dinitrobenzene was complete after 30 min and the colour intensity remained constant for a further 20 min but thereafter it decreased.

The reaction is not light sensitive and reagent blank values are low.

The quantities of the reagents used were not critical although the procedure described above is standard in our laboratory. Other solvent systems may be used successfully for diluting the reaction mixture, but 10% v/v ethanol in ethyl acetate provided maximum intensity of colour without precipitation of the reactants.

Unknown quantities of norethynodrel were assayed in an identical manner to that described above for the calibration curve. For the assay of tablets containing norethynodrel the colour was preferably compared against that produced by a similar quantity of pure norethynodrel acting as a standard. Rigorous temperature control is unnecessary in this case and reagent grade solvents and reagents may be used. The tablet excipients do not interfere with the colour development, nor does the associated mestranol, indeed no significant colouration was produced even when this substance was present in ten-fold excess over the quantity demanded by the tablet formulation.

The results obtained from 10 determinations on tablets each containing a theoretical 2·5 mg norethynodrel and 0·1 mg mestranol as active components showed a mean recovery of 2·47±0·057 (s.d.). The percentage recovery from theoretical was 98·8±2·3 (s.d.).

Investigations using other steroids have shown that this colour reaction is not restricted to norethynodrel, and preliminary results for methylandrostanolone in tablets have shown that a similar colour reaction is obtained with maximum extinction at about 560 mμ.

Results with methyltestosterone have been disappointing.

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References

Corker, C. S., Norymberski, J. K., & Throw, R. (1962). *Biochem. J.*, **83**, 583–588.