A procedure for the estimation of oestradiol, prednisone and testosterone in propylene glycol

Recently the need arose for this laboratory to develop a simple spectrophotometric method for the semi-quantitative estimation of a mixture of oestradiol, prednisone and testosterone in propylene glycol. The major problem was to isolate the testosterone; estimation of the other two ingredients presented no difficulties.

Oestradiol was isolated from a methylene chloride solution of the formulation by extraction into alkali, thorough washing with methylene chloride, acidification and re-extraction into methylene chloride. The solution was washed with water and saturated brine, dried with magnesium sulphate and made up to a known volume. An aliquot was taken, evaporated to dryness and redissolved in an equal volume of ethanol. Prepared mixtures were treated similarly. The absorbance was measured at 280 nm (Scott, 1964a) and compared with a standard solution.

Prednisone was determined by the U.S.P. XVII blue tetrazolium assay for corticosteroids, using an ethanolic solution of the mixture. It was established by means of prepared solutions that the other ingredients did not interfere with the prednisone assay.

Before the testosterone could be estimated, it had to be isolated from the other ingredients, particularly prednisone, since its λ_{max} of 238 nm (MeOH) (The Merck Index, 1968a) was identical with that of the corticoid (The Merck Index, 1968b). The solubility of testosterone in hexane was indicated by Scott (1964b), who reported a value for its absorbance in that solvent, in which prednisone is insoluble.

After removal of oestradiol from the mixture by alkaline extraction, the organic layer was thoroughly washed with water and sodium chloride solution to remove all the propylene glycol. After drying over magnesium sulphate, the solution of testosterone and prednisone in methylene chloride was evaporated to dryness on a rotary film evaporator, and the residue was allowed to form a thin film of glass-like consistency over the inner surface of the flask. A measured volume of hexane was added and the flask was swirled gently for 2–3 min, after which the extract was decanted. This was repeated eight times, with each extract being kept separate. The absorbance of each solution was measured to determine when extraction was complete. The first two extracts had absorbances greater than 2, but the eighth read only 0·03. Thin-layer chromatography [solvent; benzene–methanol (4:1): adsorbent; silica gel G, 0·25 mm] showed only testosterone (Rf = 0·65) in each of the fractions, and only prednisone (Rf = 0·50) in the residue. The extracts were combined in a volumetric flask, after pouring through a glass-wool plug, and the solution was made up to volume. The absorbance was compared with a standard solution. Similar treatment of prepared solutions showed the extraction procedure to be reproducible.

The solvent extraction procedure as described was judged to be superior to all of the TLC separations of the mixture which were attempted in an effort to isolate the testosterone.

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March 11, 1970

REFERENCES

SCOTT, A. I. (1964). *The Ultraviolet Spectra of Natural Products*, (a) p. 420, (b) p. 394. New York: Macmillan Company.

The Merck Index (1968). Eighth edition, (a) p. 1020, (b) p. 862. Rahway, N.J.: Merck & Co.