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SIMULTANEOUS ESTIMATION OF TESTOSTERONE, PROGESTERONE AND ANDROSTENEDIONE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY WITH A SINGLE ION DETECTION CORRELATION WITH RADIOIMMUNOASSAY

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SUMMARY

Testosterone, progesterone and androstenedione can be estimated simultaneously in a single ether extract of plasma, using the gas chromatography–mass spectrometer combination with single ion monitoring. The quantitated ions are the molecular ions of testosterone-3-heptafluorobutyrate-17β-acetate (M^+ = 526), progesterone-3-heptafluorobutyrate (M^+ = 510) and androstenedione-3-heptafluorobutyrate (M^+ = 482). The lower limit of detection of these three hormones is in the range of 0.20–0.25 ng per ml plasma. Good correlation is obtained with the radioimmunoassay results.

INTRODUCTION

The method for the estimation of testosterone and progesterone has been published recently in this journal [1]. We have completed this method by the quantitative determination of androstenedione (4-androstene-3, 17-dione) in plasma samples, which are processed and analysed as described previously[1].

The total internal standard is 4-methyl-19-nor-androstene-3,17-dione and the quantitated ions are the molecular ions of androstenedione-3-HFB (17-oxo-3,5-

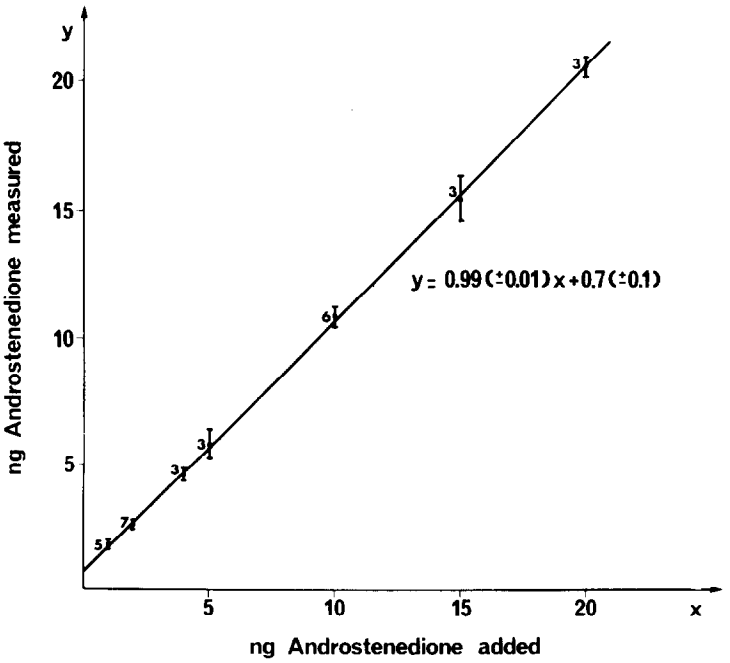

Fig. 1. Accuracy of the estimation of androstenedione (the figures on the straight line represent the number of determinations).

androstadienyl-3-heptafluorobutyrate) and 4-methyl-19-nor-androstenedione-3-HFB (4-methyl-17-oxo-3,5-estradienyl-3-HFB) with $M^+ = 482$.

The accuracy of the method (Fig. 1) was evaluated by measuring known amounts of androstenedione added to 1 ml of plasma from a pool, which contained originally 0·7 ng/ml androstenedione.

The precision of the method is given in Table 1.

The limit of detection is 0·21 ng/ml.

Table 1. Standard deviation and coefficient of variation for various androstenedione determinations

Range	0–2·0 ng/ml
Mean	1·20 ng/ml
S.D.	0·09 ng/ml
C.V.	8

REFERENCES

1. Dehennin L., Reiffsteck A. and Schöller R., *J. steroid Biochem.* **5** (1974) 81–86.

DISCUSSION

Siekmann:

With respect to your internal standard material, I would like to mention that we did not use deuterated standards for testosterone. For example, we used the commercially available tritiated material. If you work in this range of picogram amounts or even nanogram amounts, you could use tritiated material. About 1 μCi of tritiated material would be about 5–7 n and I think this is not a question of money.

Dehennin:

When one can avoid the utilization of radioactive isotopes, one should try to do it. As we have a single ion detector, we must use internal standards which are separated from the estimated hormones on the gas chromatographic column.

Siekmann:

But what about the availability of the compounds you use as internal standards.

Dehennin:

These are very easily synthesized by alkylation on the C-4 position.