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SHORT COMMUNICATION

QUANTITATION OF UP TO 12 ESTROGENS IN 1–50 μl OF PREGNANCY URINE

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SUMMARY

Mass fragmentographic studies of estrogens in urine samples in pregnancy revealed that a sensitivity from at least 100 up to 10,000 times as compared to conventional gas chromatography with hydrogen flame ionisation detector can be achieved. The specificity of the determination is also considerably increased. Even amounts less than 10 pg can sometimes be detected and analysed if optimal conditions are employed. This means that 9 estrogens may be analysed in a 1–5 μl sample of late pregnancy urine and that only about 50 μl is needed to estimate all 12 estrogens included in the method. Mass fragmentograms of 12 naturally occuring estrogens are presented.

INTRODUCTION

Only a few methods for the quantitative determination of estrogens other than the three "classical ones" estrone, estradiol, and estriol are available at present [1–3]. They have all been developed for pregnancy uring and only one of them has been used for the analysis of estrogens in other biological fluids and tissues [4, 5]. However, samples of biological fluid as large as 100–200 ml or up to 200 g of tissue are needed when analysing fluids and tissues other than urine.

Development of the single and multiple ion detection techniques in combined gas chromatography-mass spectrometry has made the quantitation of steroids and other compounds in the picogram range possible. Recently, two reports on the use of this technique in the analysis of steriods in biological material have been presented [6, 7]. Quantitative methods for the determination of 15α-hydroxyestriol in urine and of testosterone in plasma based on mass fragmentography have also been presented [8, 9].

The present report deals with some mass fragmentographic analyses of estrogens in urine during pregnancy.

EXPERIMENTAL

Pregnancy urine samples were processed using the method of Adlercreutz and Luukkainen [3], the details of which, including some modifications, will be reported elsewhere [10]. All fractions were first analysed by gas chromatography (g.c.) in order to obtain information on the exact quantities of various estrogens present. Thereafter they were diluted to concentrations suitable for mass fragmentography and analysed in a VARIAN MAT CH7 combined gas chromatograph-mass spectrometer with a multiple ion detector. When especially high sensitivity was required single ion monitoring was used.

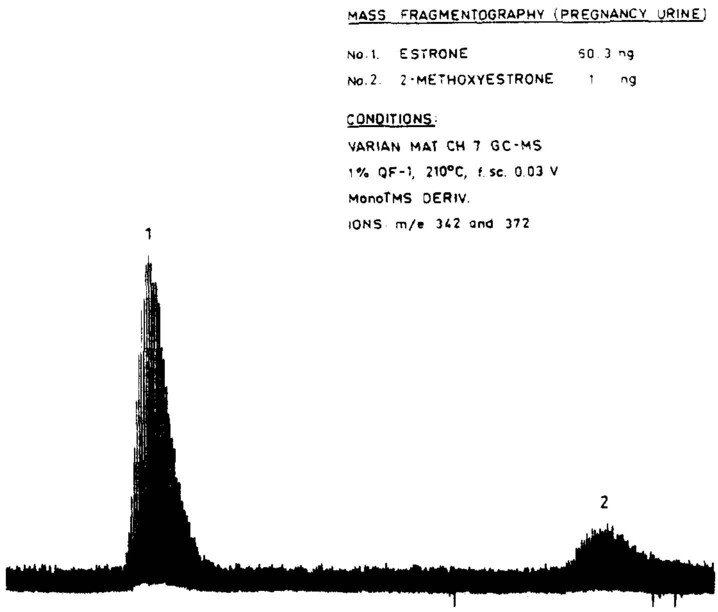

Fig. 1. Mass fragmentogram of the estrone-2-methoxyestrone fraction obtained from normal pregnancy urine. The analytical conditions are shown in the figure. Abbreviations: g.c.-m.s. = Gas chromatograph-mass spectrometer; f.sc. = full scale; MonoTMS deriv. = Monotrimethylsilyl ether derivative.

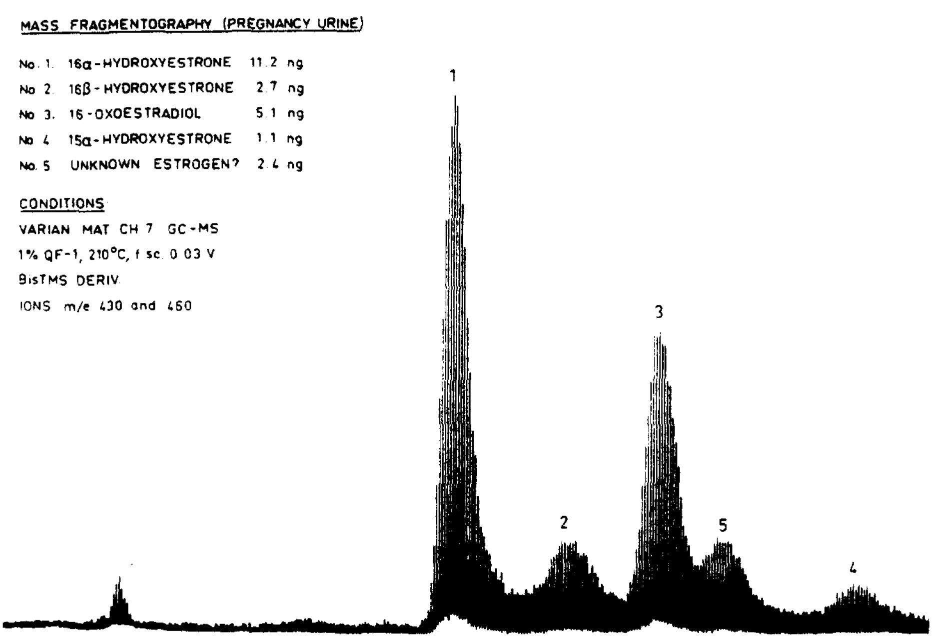

Fig. 2. Mass fragmentogram of the Ring D-ketolic estrogen fraction obtained from normal pregnancy urine. For explanations see text to Fig. 1.

RESULTS AND DISCUSSION

Some of the fragmentograms obtained from urine samples at various concentration levels (50 pg–50 ng) are shown in Figs. 1–5. The ultimate sensitivity of the VARIAN MAT CH7 was tested using urinary estriol measurement as the parameter. With the multiple ion detection system 5 pg of estriol as its tritrimethylsilyl ether derivative gave a peak 2·4 cm high, using the single ion monitoring the same response was obtained with 2·1 pg of estriol. Both peaks were 6–7 times greater than the background noise range. There still exist certain possibilities to enhance the sensitivity of this technique. For some of the compounds studied a higher sensitivity could perhaps have been achieved by choosing a g.c.-column with less background noise. However, in this study we used those columns which were employed in the original g.s. procedure and which, with that method, gave the most specific results. Comparison of the gas chromatograms and the mass fragmentograms (several ions were monitored for each fraction) revealed that the specificity of the determination is better when mass fragmentography is used. However, the present analyses also demonstrated that only very little contamination, steroid or otherwise are present in the estrogen peaks obtained with the original procedure. A detailed computer analysis of all fractions will be published elsewhere (Adlercreutz and Hunneman).

The studies also revealed, as can be seen from the mass fragmentograms, that without special effort it is possible to quantitate 9 estrogens in 1–5 μl of pregnancy urine at term and that only about 50 μl is needed for the determination of all 12 estrogens. The concentration range for the various estrogens in the last trimester of pregnancy is about 30–25,000 pg/μl of urine[11] as determined by the original procedure. It is likely that the tedious purification procedure

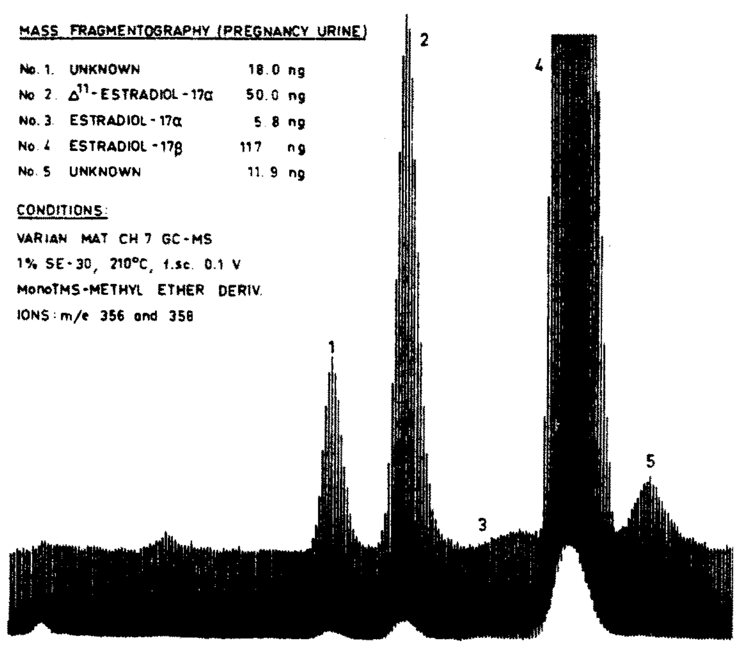

Fig. 3. Mass fragmentogram of the estradiol fraction obtained from normal pregnancy urine. For explanations see text to Fig. 1.

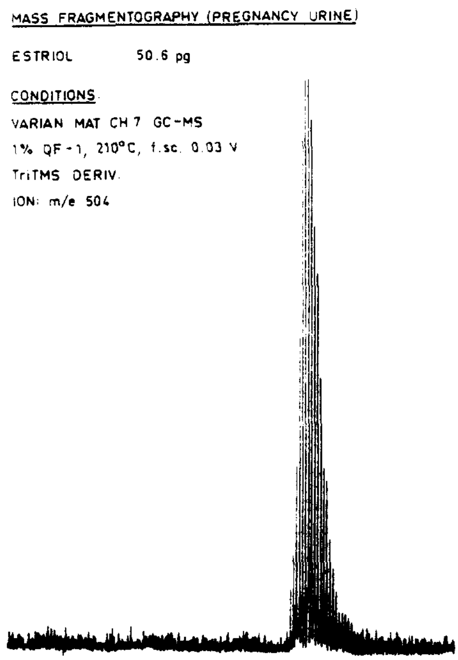

Fig. 4. Mass fragmentogram of the estriol fraction obtained from normal pregnancy urine. For explanations see text to Fig. 1.

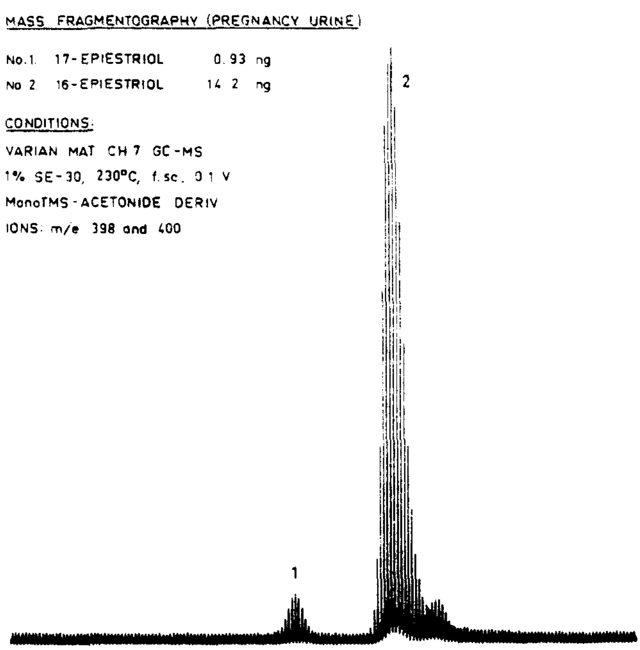

Fig. 5. Mass fragmentogram of the 16-epiestriol-17-epiestriol fraction obtained from normal pregnancy urine. For explanations see text to Fig. 1.

can now be considerably shortened as a result of the high specificity and sensitivity of the new fragmentographic technique.

The results seem promising and indicate that not more than about 1 ml of plasma is needed for the determination of these estrogens in late pregnancy. Recent preliminary studies suggest that about 1–2 μl of plasma is enough for the quantitation of unconjugated estriol and that this can be done without prior chromatography. The sensitivity of the technique, which is sometimes better than that obtained using radioimmunoassay, combined with the possibility to analyse many compounds simultaneously will make it essential for analysis of small tissue samples. Thus it will surely constitute one of the most powerful analytical research tools in the near future.

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