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Note

Malachite green —a new staining reagent for prostaglandins

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The determination of prostaglandin (PG) E compounds in human seminal fluid is of clinical interest since it has been suggested that the prostaglandins are of importance for normal fertility[1]. Bygdeman *et al.*[2] have described a method for quantitative determination of prostaglandin E and F compounds in human seminal fluid.

There are various staining reagents[3,4] used for the detection of prostaglandins on thin-layer chromatography (TLC) plates. In this communication malachite green is described as a new staining reagent for the detection of prostaglandins on either silica gel glass fiber paper or TLC.

MATERIALS AND METHODS

The preparation of silica gel glass fiber paper, the extraction of prostaglandins and the separation of prostaglandins have been described earlier[4,5]. The TLC of prostaglandins was described elsewhere[2].

Standard and unknown samples (seminal prostaglandins) in ethanol were applied as spots 1–2 mm in diameter on silica gel glass fiber paper. The chromatogram was developed by the ascending method in a tightly closed rectangular tank. The chromatogram was developed over a distance of 15 cm with the solvent ethyl acetate–water–methanol (110:20:1), equilibrated overnight before the organic layer was used. After drying, the chromatogram was evenly sprayed with a 0.1 % solution of malachite green and heated at 80° for 10 min. The density of the spots was determined by a densitometer equipped with a recorder. The color intensities of the spots can also be semiquantitatively estimated visually.

RESULTS AND DISCUSSION

The sequence of separation is: PGB (R_F 0.87) —PGE_1 (R_F 0.59)— PGE_2 (R_F 0.46) —$PGF_{2\alpha}$ (R_F 0.35)— $PGF_{2\alpha}$ (R_F 0.21). The malachite green spray gives these characteristic colors: Blue for prostaglandin F and E compounds; bluish green for prostaglandin A and B compounds. The staining reagent is sensitive, giving excellent results with 0.3–0.5 μg of prostaglandins. The spots obtained with the reagent are stable for several days. On a TLC plate the malachite green staining reagent is less sensitive as compared with silica gel glass fiber paper. The sensitivity of the spot on TLC is approximately 0.7–1.0 μg. Using malachite green as a staining

reagent is a very quick method for the detection and estimation of prostaglandins. The reagent is stable for several months. Any clinical laboratory can develop this technique for routine analysis of prostaglandin E compounds in human semen since it is reported that prostaglandin E compounds are linked to fertility in the male.

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