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A simple staining technique for the detection of steroids on silica-gel thin-layer glass plates

In general, the detection of various ultraviolet non-absorbing steroids on silica-gel thin-layer glass plates is achieved by first spraying parts of the chromatogram with a suitable reagent or reagents, followed by heating the plate for several minutes at 70–100° [1,2]. This procedure has been used widely and one of the most commonly used spray reagents for the detection of steroids and sterols is a 2–20 % solution of molybdophosphoric acid in ethanol[2–4]. Most of the steroids develop a molybdenum blue colour after heating. In the standard procedure for analytical or preparative thin-layer chromatography, the crude sample is applied along a line in the middle of the silica-gel plates and on the two sides are applied authentic marker steroids. After development in a suitable system, the chromatogram is dried and the marker steroid lanes are sprayed with a 10 % ethanolic solution of molybdophosphoric acid, and the plate is then heated. This procedure has two main disadvantages: the spraying of the reagent has to be confined only to the marker steroid lanes and in many instances the reagent crosses over into the area of the sample being purified, resulting in losses; and the heating of the plate may result in decomposition of steroids present in the sample being purified, particularly in the case of labelled steroids having a high specific activity. A simple technique is described in this paper that enables various steroids to be detected without the plates being heated.

The technique consists of applying and chromatographing the unknown sample and authentic steroids as in the manner described above. After chromatography, a 2-mm wide portion of the marker steroid lane is streaked with molybdophosphoric acid (10%) with a Pasteur pipette. The plate is left in the air for a few minutes at room temperature and then concentrated sulphuric acid is applied in the same manner to the area stained with molybdophosphoric acid, taking care to confine the sulphuric acid only to areas stained previously with molybdophosphoric acid. Immediately after the sulphuric acid treatment, the area containing steroids shows up as dark blue spots on a light green background. The blue colour produced is quite stable and does not fade significantly for several hours.

Thus, in this procedure spraying and heating are not involved and the unknown sample spotted in the middle of the plate can be recovered by the usual procedures[2]. The blue colour obtained is much darker for the same amount of steroid than that obtained with molybdophosphoric acid followed by heating of the plate. The specificity of this test is the same as that with molybdophosphoric acid alone, although the sensitivity is higher. The smallest amounts of steroids that can be detected by this procedure have not been determined, but 5 $\mu g/cm^2$ for most steroids was sufficient for detection by this procedure. It should be noted that this method is not applicable to ready-made (Eastman Chromagram 6060) plates made of silica gel containing fluorescent indicators, as a very dark background is obtained.

Some representative steroids and sterols that have been detected by this procedure are cholesterol, lanosterol, 15α-hydroxypregnenolone, 16α-hydroxypregnenolone, pregnanediol, dehydroisoandrosterone, 5α-androstane-3α,17β-diol, androsterone, etiocholanolone, dehydroisoandrosterone acetate, pregnenolone sulphate, oestrone, oestra-

diol and oestriol. The ultraviolet-absorbing steroids such as progesterone, cortisol, testosterone and androstenedione can also be detected by this method, but the intensity of the blue colours obtained with these steroids is very weak.

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