A new method for determining alkaloids of the atropine group by thin-layer chromatography

Although many methods of TLC and PC[1,2,3] have been applied to the separation of tropane alkaloids, few reports have described a rapid and simple method. Of the methods employed so far, even the best ones reported by STAHL and RANDERATH require alkalinization or impregnation of the layer. When this layer is a formamide impregnated cellulose, the experiment is prolonged by the necessity of having to eliminate the formamide after the development of the chromatogram, either by heating it in a vacuum or by a chemical procedure. In the course of our present study of the distribution and biogenesis of alkaloids in *Hyoscyamus muticus* and *Hyoscyamus aureus* L.[4], we have perfected a simple technique for the evaluation of the content of alkaloids of the atropine group by thin-layer chromatography. A successful separation with sharper spots is simply and quickly obtained by using:

(A) Glass plates with a cellulose adsorptive layer developed in the new solvent system isobutanol–fuming HCl–H_2O (7:1:2 v/v).

(B) The detection of the spots with the Dragendorff reagent modified by TRABERT[5], and the quantitative determination of the eluent by spectrophotometry.*

Materials and methods

15 g of Whatman cellulose without binder (Chromedia CC41) are homogenized in 45 ml distilled water and spread on five 10 × 20 cm glass plates with a thin-layer applicator (*e.g.* Desaga) at a thickness of 0.25 mm. After air-drying the plates are activated at 110° for 30 min, and 1–10 μl of methanolic solutions of hyoscyamine hydrobromide, scopolaminium bromide (Merck), N,N,N′,N′-tetramethylbutane-1,4-diamine (Koch-light Laboratories), and alcohol extracts of the two species of *Hyoscyamus* are spotted. The aqueous phase of the system is used to saturate the glass tank and the organic phase acts as solvent. One and three quarter hours is sufficient

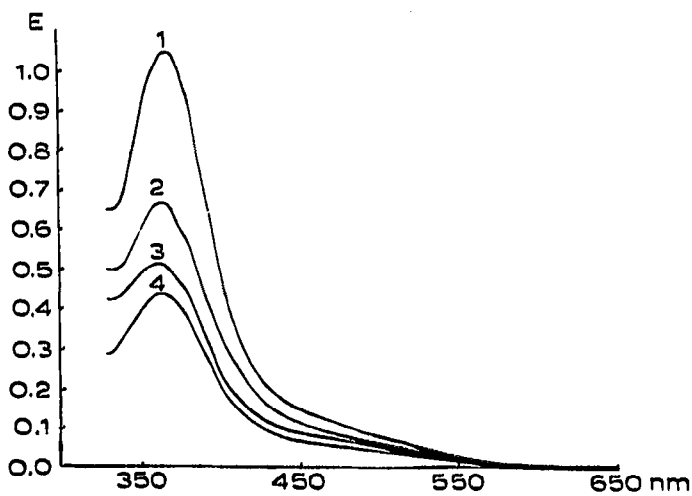


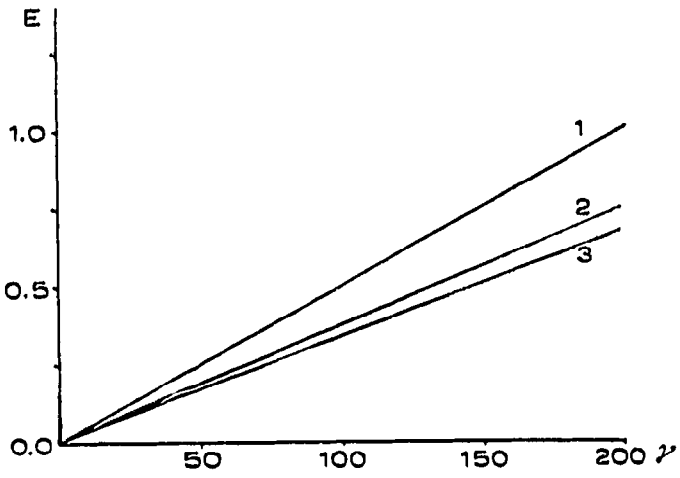
Fig. 1. Spectrophotometric absorption curves of: (1) N,N,N′,N′-tetramethylbutane-diamine, (2) scopolaminium bromide, (3) hyoscyamine, and (4) blank.

* We used the Cary spectrophotometer for our measurements.

for a migration of 12 cm. Alkaloid spots are detected by spraying with the Dragendorff reagent modified by TRABERT[5]. The plates are air-dried overnight in the dark. The red-orange spots of the alkaloid complex are marked, scraped off with a razor blade, and eluted with acetone. After complete decoloration, the powder and eluent are sucked up by means of an aspirator (GORBACH type modified by us)[6], the powder is stopped on a porous glass disc and the eluent is extracted with a vacuum pump into a volumetric flask, adjusted to 5 or 10 ml, and evaluated with the spectrophotometer. In Fig. 1 the spectra obtained are given.

It is important to note that the extinction is stable even after several days if the solution is kept in the dark.

The linear relationship between the concentration of the spot and the extinction is shown in Fig. 2.



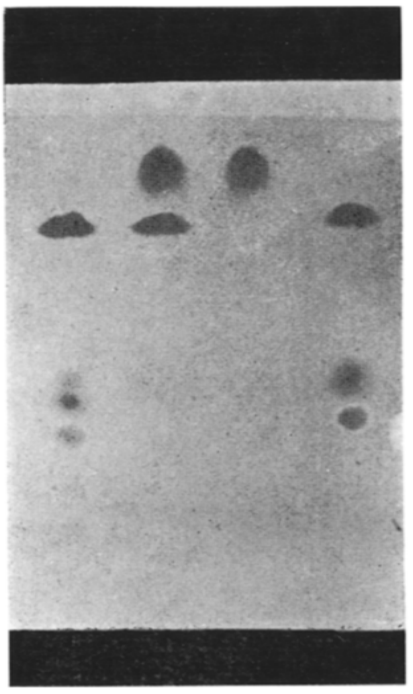


Fig. 2. Linear relationship between the concentration of the spot and the extinction (the numbers 1, 2, 3 have the same significance as in Fig. 1).

Fig. 3. TLC of hyoscyamine (atropine), scopolaminium, N,N,N′,N′-tetramethylbutane-diamine on cellulose layer developed in an isobutanol–fuming HCl–H_2O (7:1:2, v/v) solvent system. Detection with the Dragendorff reagent modified by TRABERT[5].

Discussion

This very simple technique, requiring no chemical treatment, impregnation, or alkalinization etc.[1,2] of the cellulose layer, has two advantages over the method of determination after paper chromatography[3] used previously:

(1) Economy of time: 1 h and 45 min with thin-layer chromatography instead of 20 h,

(2) Sharper spots and a clearer separation due to the very small diffusion capacity of the cellulose layer and its greater selectivity.

It is necessary, however, to control the degree of humidity of the cellulose

powder as this influences the separation of the various alkaloids. It can be modified either by varying the proportion of water (45 to 40 ml H_2O for 15 g Chromedia CC 41), or more efficiently, by carrying out successive uni-dimensional developments of the chromatogram in the same system and up to the same level[7].

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