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Short Communication

Determination of some narcotic and toxic alkaloidal compounds by overpressured thin-layer chromatography with ethyl acetate as eluent

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ABSTRACT

A good separation of the components of various chemical classes of alkaloids is possible by overpressured thin-layer chromatography (OPTLC) on aluminium oxide plates with ethyl acetate alone as the mobile phase. The OPTLC method is efficient, rapid (15 min) and combines the advantages of classical TLC and HPLC, *i.e.*, large numbers of samples, high resolution and speed and the use of selective reagents. It is possible to separate the most commonly used narcotic and toxic agents with this method. For cannabinoids (narcotic phenolic compounds), the characterization requires the use of a binary eluent [hexane-ethyl acetate (70:30, v/v)].

INTRODUCTION

Toxicological laboratories are constantly engaged in studies of toxic and narcotic substances from different sources (powders, cigarettes, syringe residues, other pharmaceutical forms, etc.). Alkaloids are the most important group of narcotic and toxic agents and methods for the rapid detection of different classes of these compounds and their derivatives are required. Overpressured thin-layer chromatography (OPTLC) can be used for this purpose, as was demonstrated previously for the separation of alkaloids [1,2].

As a continuation of our investigations and the development of the process, we have applied OPTLC to the determination of the most frequently used narcotic and toxic agents such as lysergide (LSD), heroin, cocaine, amphetamine, nicotine and opium alkaloids such as morphine and codeine.

EXPERIMENTAL

Apparatus

The samples were spotted with Minicaps (Hirschmann Laborgeräte, Eberstadt/Heilbronn, Germany). Chromatography was performed with 20 x 20 cm aluminium oxide 60 F_{254} Type E 5713 precoated glass plates (Merck, Darmstadt, Germany). The plates were impregnated on three sides with Impress No. II polymer suspension (Laborate, Budapest, Hungary).

OPTLC was performed with a Chrompres 25 chromatograph (Laborate). During chromatog-

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raphy, the external pressure of the water cushion was 15 bar, the starting mobile phase (ethyl acetate) pressure was 7 bar and the flow-rate was 0.30 ml/min. The development of the plates (14 cm length) took 15 min.

Densitograms were recorded with a Camag Model 76510 TLC/HPTLC scanner at 540 nm after detection with Dragendorff's reagent.

Preparation of standard mixture and solutions

All solvents were of analytical-reagent grade from Merck. Before use, ethyl acetate was filtered through a 0.45-μm Millipore membrane after sonication.

A standard mixture was prepared by dissolving 10 mg of each of the following reference substance in 1 ml of methanol: LSD (Sandoz), heroin, cocaine, amphetamine, nicotine, morphine and codeine. The sample of opium was a tincture obtained by maceration for 24 h of 1 g of opium powder in 10 ml of 60% ethanol.

The volume of the spots applied on the chromatographic plates was 2 μl, corresponding to 20 μg for each sample. After elution the plates were sprayed with Dragendorff's reagent as modified by Munier and Macheboeuf [3] or potassium iodoplatinate reagent [4], which gave characteristic shades for the different spots (Table I), then read with the densitometer.

A 0.1-μg amount of cannabis resin was extracted by shaking at room temperature for 20 min with 10 ml of hexane, the filtrate was evaporated to dryness and the residue dissolved in 1 ml of toluene. A 20-μl volume of the toluene solution was spotted on the plate.

Standard solutions of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabinol were obtained by dissolving 1 mg of each in 5 ml of toluene. Volumes of 20 μl of the cannabis resin extract, corresponding to 2 μg, and 2 μl of the Δ^9-THC and cannabinol solutions, corresponding to 0.4 μg, were spotted on the chromatographic plates.

For cannabinoids, Fast Blue B Salt reagent was used as the spray reagent [5].

RESULTS AND DISCUSSION

The most interesting result of this work is the separation of alkaloidal drugs with use of only ethyl acetate as the mobile phase. This is a significant improvement because usually in TIC and OPTLC systems three or more components in the mobile phase are required [6–11]. Such mixtures of eluents are not easy to use with basic components such as ammonia and diethylamine, which can both affect detection by visualization and determination by densitometry. The use of a single solvent also ensures good reproducibility of the method.

The procedure is also interesting because the

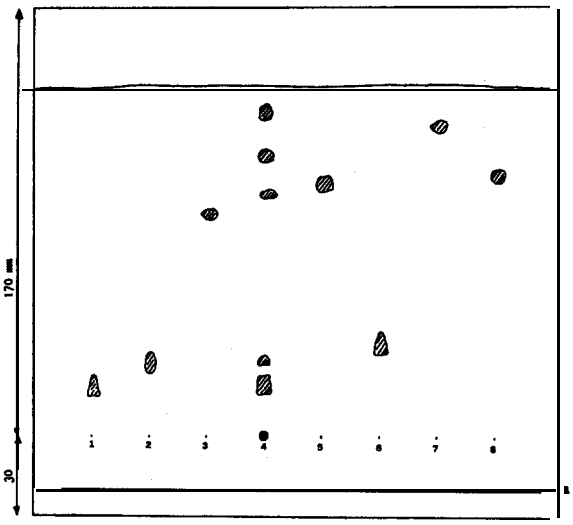

Fig. 1. Chromatogram of some narcotic and toxic alkaloids. 1 = Morphine; 2 = codeine; 3 = heroin; 4 = opium alkaloids; 5 = nicotine; 6 = amphetamine; 7 = cocaine; 8 = LSD. a = Start of elution.

TABLE I

hR_F VALUES OF THE DIFFERENT ALKALOIDS AND THE COLOURS OBTAINED WITH IODOPLATINATE AND DRAGENDORFF'S REAGENTS

Alkaloid	hR_F	Dragendorff's reagent	Iodoplatinate reagent
Amphetamine	20	Yellow-orange	Light pink
Codeine	26	Orange	Pink-violet
Cocaine	92	Orange	Violet
Heroin	61	Orange	Yellow
LSD	67	Brown	Pink-brown
Morphine	13	Orange	Deep blue
Nicotine	77	Red-orange	Black-blue
Noscapine	95	Orange	Pink-brown
Papaverine	82	Orange	Light pink
Thebaine	72	Orange	Brown-violet

different compounds studied are cleanly separated. The chromatogram of opium alkaloids (Fig. 1) shows an efficient separation which permits the identification of all major alkaloids, which is not always the case in the TLC separation of alkaloids.

For other narcotic and toxic agents we obtained good separations. A list of the standards studied and their hR_F values are given in Table I.

The densitogram obtained from a standard mixture containing LSD, heroin, cocaine, amphetamine nicotine and the principal opium alkaloids morphine and codeine (Fig. 2) shows a clean separation of the different compounds after detection by dipping in or spraying with Dragendorff's reagent. Detection with iodoplatinate reagent gives different characteristic shades, which permits rapid identification (Table I).

It was observed that it is often possible for there to be a slight difference in migration if standards are spotted alone or in mixtures. These differences were observed particularly for codeine and LSD (Fig. 1). However it is easy to determine the nature of the compounds by the specific coloration developed with the different reagents used for spraying the plates (Table I).

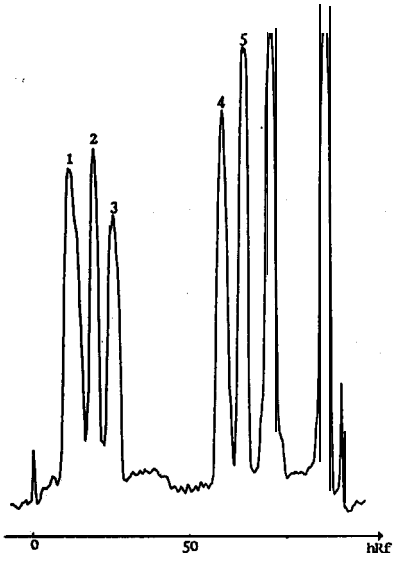

Fig. 2. Densitogram of standard mixture. 1 = Morphine; 2 = amphetamine; 3 = codeine; 4 = heroin; 5 = LSD; 6 = nicotine; 7 = cocaine.

With ethyl acetate as the mobile phase, Δ^9-*THC* and other cannabinoids (narcotic phenolic compounds) from ***Cannabis sativa var. indica*** migrate up to the solvent front, but it is possible to separate them by reducing the mobile phase polarity by addition of hexane [hexane-ethyl acetate (70:30, v/v)]. The characterization of these compounds is as usual with Fast Blue B salt reagent. In this instance a good separation of cannabinoids is obtained, particularly of cannabinol, which is found in all cannabis species, and Δ^9-THC, which is the major active euphoric principle. The hR_F values are 82 for cannabinol and 77 for Δ^9-THC.

CONCLUSIONS

The method reported is efficient, reproducible and rapid (15–20 min per analysis). In comparison with the TLC of alkaloidal compounds under the same experimental conditions, OPTLC with ethyl acetate as the mobile phase gives a better resolution with longer development distances and the sensitivity is better because there is less diffusion of the compounds than in TLC. The same applies to the separation of cannabinoids with hexane-ethyl acetate as the mobile phase.

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