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Short Communication

Separation of opium alkaloids by thin-layer chromatography combined with flame ionization detection using the peak pyrolysis method[a]

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ABSTRACT

A fast, efficient and reproducible method for the separation of the five major opium alkaloids by thin-layer chromatography coupled to flame ionization detection is reported. The alkaloids were separated without derivatization on silica rods of type SII using the partial scanning or peak pyrolysis method —where organic matter separated on the rods are burnt when they pass through the hydrogen flame— between the stages of a two-step development system. The first development, in benzene–acetonitrile–ethyl acetate (60:20:20, v/v) separated narcotine and papaverine. Morphine, codeine and thebaine were separated during the second development in ethyl acetate–benzene–acetonitrile–ammonium hydroxide (25:30:40:5, v/v). The best binary solvent system was benzene–ethanol (9.5:0.5, v/v) and (9:1, v/v) which separated all the five alkaloids simultaneously.

INTRODUCTION

The separation of opium alkaloids is of prime importance in toxicology and forensic and pharmaceutical chemistry. Separations of alkaloids by different chromatographic methods were reviewed by Giplin and Pachla [1,2]. Borke and Kirsch [3] were the first to report the separation of opium alkaloids by thin-layer chromatography (TLC). Misra *et al.* [4] used glass-fibre sheets to separate morphine, codeine and thebaine and some of their metabolites and congeners. Two-dimensional TLC was used by Munier and Meunier [5] to separate five alkaloids on a silica gel plate. Okumura *et al.* [6] separated some opium alkaloids on silica gel sintered sticks, but the sticks had to be rechromatographed to remove excess of diethylamine, which was used as one of the solvents. Recently, Patzch *et al.* [7] have used high-performance

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TLC to determine morphine, codeine and heroin on silica gel plates with post-column derivatization.

This paper reports a rapid, relatively simple and reproducible method for the separation of the five major opium alkaloids, *viz.*, morphine, codeine, thebaine, papaverine and narcotine (noscapine). TLC coupled with flame ionization detection (FID) combines the efficiency of TLC and the sensitivity of FID. It has the added facility allowing the use of the partial scanning or peak pyrolysis method [8]. Analysis by TLC–FID does not require derivatization of samples and high operating temperatures as in gas chromatography or the use of large volumes of solvent and maintenance of pH as in high-performance liquid chromatography. As little as 2–3 μg of each compound is needed for detection without derivatization.

EXPERIMENTAL

Apparatus

An Iactroscan TH-10 MK III analyser (Iatron Labs., Tokyo, Japan) was equipped with a flame ionization detector and connected to a two-pen linear recorder (National).

Materials

The alkaloids morphine, codeine, thebaine, papaverine and narcotine were obtained from Government Opium and Alkaloids Works Undertaking (Neemmuch, M.P., India).

Procedure

The detector was operated with a hydrogen flow-rate of 160 ml/min and an air flow-rate of 2000 ml/min. The recorder was used at 50–100 mV full-scale deflection. The chart speed was kept at 12 cm/min and the scanning speed at 35 s per scan. A new set of Chromarod SII silica rods (particle size 5 μm) (Newman–Howells, UK) was used throughout.

The alkaloids were dissolved in methanol–dichloromethane (2:1, v/v). Spotting was done in aliquots of 0.25 μl to prevent spreading of the sample on the rod. Calibration was done by spotting different volumes (0.5–3 μl) of standard samples of the same concentration (2 mg/ml). Absolute calibration graphs were drawn for each standard sample as the peak area (abscissa) *versus* spot weight (ordinate). The calibration graphs were linear and the slope was determined using the equation $y=mx$ ($y=$ spot weight in mg; $x=$ peak area and $m=$ slope). The slopes were thebaine = 1, morphine = 0.99, codeine = 0.99 and narcotine = 1.2. The graphs were linear from 1 to 7 μg. As long as analysis and calibration are performed under identical conditions, the calibration graphs can be used to determine directly the weights (in μg) of the components provided that the peak areas of the components are known. When 1 μl of a sample containing a mixture of the alkaloids of concentration12.4 mg/ml was spotted and the peak areas for the different components were determined, the amounts (in μg) could be read directly from the calibration graph.

The alkaloids were separated using the partial scanning method between the stages of a two-step development system; details are given under *Results and Discussion.* Narcotine and papaverine were separated using benzene–acetonitrile–ethyl

TABLE I

R_F VALUES OF OPIUM ALKALOIDS ON CHROMAROD SII SILICA RODS

Solvent system	Composition (%, v/v)	Mode of development	R_F value				
			Morphine	Codeine	Thebaine	Papaverine	Narcotine
Benzene–acetonitrile–ethyl acetate	60:20:20	Two-step development system with peak pyrolysis in between	0.13	0.15	0.18	0.41	0.56
Ethyl acetate–benzene–acetonitrile–ammonia solution	25:30:40:5	As above	0.24	0.33	0.49	–[a]	–[a]
Benzene–ethanol	9.5:0.5 then 9:1	Dual development	0.16	0.23	0.38	0.63	0.70

[a] Burnt during peak pyrolysis.

acetate (60:20:20, v/v) as solvent system in the first step and morphine, codeine and thebaine using ethyl acetate–benzene–acetonitrile–ammonia solution (25:30:40:5, v/v) in the second step.

Dual development with benzene–ethanol (9.5:0.5 and 9:1, v/v) effected the simultaneous separation of the five opium alkaloids.

RESULTS AND DISCUSSION

The R_F values of five major alkaloids on Chromarod SII silica rods are given in Table I. Binary and multi-component solvent systems involving one- or two-step development systems were employed. The best multi-component solvent system involving peak pyrolysis between two-step development and a binary system which effects the separation of all five opium alkaloids simultaneously is reported. Results obtained by quantitative analysis are given in Table II.

TABLE II

QUANTITATIVE ANALYSIS OF A MIXTURE OF OPIUM ALKALOIDS

Alkaloid	Peak area[a]	Amount applied (μg)	Amount found (μg)[b]	Recovery (%)	Standard deviation of peak area[a]
Narcotine	52.4	3.77	3.75	99.4	3.0
Thebaine	41.1	2.12	2.10	99.05	4.7
Codeine	68.7	3.01	3.00	99.6	4.3
Morphine	77.9	3.51	3.50	99.7	1.8

[a] Mean of six determinations.

[b] From calibration graph.

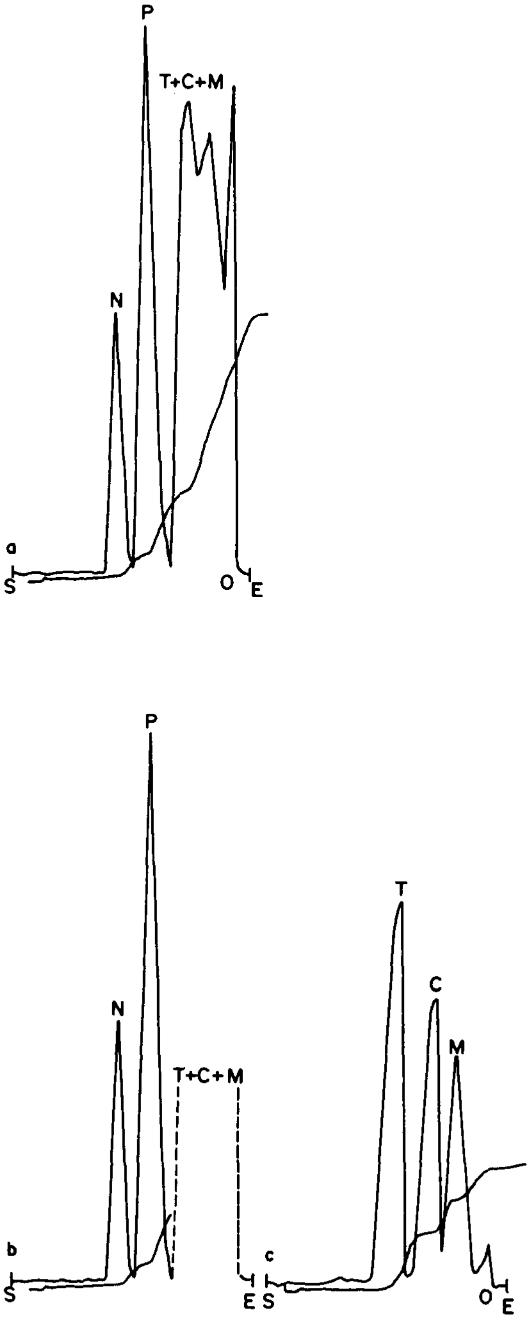

Fig. 1. Two-step development for separation of opium alkaloids on Chromarods SII. (a) Complete scan of the five opium alkaloids developed with benzene–acetonitrile–ethyl acetate (60:20:20, v/v); (b) partial scan; (c) development with ethyl acetate–benzene–acetonitrile–ammonia solution (25:30:40:5, v/v) resolved the forked peak into morphine, codeine and thebaine. P = Papaverine; N = narcotine; T = thebaine; C = codeine; M = morphine.

The fastest migrating alkaloid was narcotine, followed by papaverine, thebaine, codeine and morphine. This pattern of elution was constant for both binary and multi-component systems. It should be noted that in the figures the direction of development is from right to left and the scanning direction is from left to right. Hence, narcotine, which is the fastest moving compound, elutes as the first peak and morphine, which is very sluggish, elutes as the last peak. S and E denote the start and end of the scans, respectively, and **O** is the point of sample application.

The quaternary system suggested by Steele [9] was investigated. This system consists of ethyl acetate–benzene–acetonitrile–ammonia solution in different ratios such as (a) (50:30:15:15, v/v) and (b) (25:30:40:5, v/v). System (a) gave only a moderate separation of morphine, codeine and thebaine and no separation of narcotine and papaverine. However, system (b) resolved the peaks of morphine, codeine and thebaine well, but there was no change in the separation of papaverine and narcotine. We therefore concluded that a less polar system such as benzene–acetonitrile–ethyl acetate (60:20:20, v/v), might give a clean separation of narcotine and papaverine. Hence, a two-step development sequence with the partial scanning or peak pyrolysis method could be a successful approach to this problem.

The first development was done with benzene–acetonitrile–ethyl acetate (60:20:20, v/v). This resulted in the fastest migrating compounds, *i.e.*, narcotine and papaverine, to migrate into the upper part of the rod, morphine, codeine and thebaine remaining near the point of application and appearing as a forked peak, as can be seen in Fig. 1a. The scan stop screw was then set at a point between the resolved and unresolved compounds and the remaining rods were partially scanned, as shown in Fig. 1b. In this way, narcotine and papaverine were determined and this part of the rod was reactivated so that a second development could be performed with a more

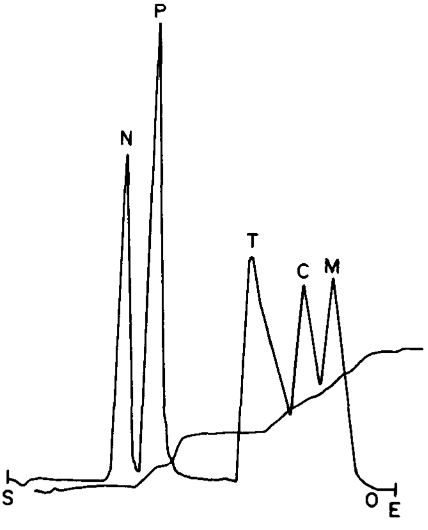

Fig. 2. Simultaneous separation of the five major alkaloids of opium on Chromarods SII developed twice with benzene–ethanol (9.5:0.5 and 9:1, v/v). P = papaverine; N = narcotine; T = thebaine; C = codeine; M = morphine.

polar solvent system, taking the unresolved compounds (morphine, codeine and thebaine) as the new origin. The second development of the rods was done with ethyl acetate–benzene–acetonitrile–ammonia solution (25:30:40:5, v/v), which gave a good separation of morphine, codeine and thebaine (Fig. 1c). The rods in both these systems were developed up to 10 cm for a period of 35 min each and scanned at 35 s per scan. A dual development with a binary system consisting of benzene–ethanol (9.5:0.5 and 9:1, v/v) effected the simultaneous separation of all the five alkaloids (Fig. 2).

CONCLUSION

All five major opium alkaloids could be separated without derivatization on Chromarod SII silica rods using partial scanning or peak pyrolysis between the stages of a two-step development system. The first development with benzene–acetonitrile–ethyl acetate (60:20:20, v/v) helps to move the less polar compounds away from the point of application. The remaining unresolved compounds are then separated with a more polar solvent system, ethyl acetate–benzene–acetonitrile–ammonia solution (25:30:40:5, v/v). The best binary solvent system was benzene–ethanol (9.5:0.5 and 9:1, v/v), which could separate all five alkaloids simultaneously.

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