CHROM. 18 805

Note

Simultaneous separation of the principal alkaloids in gum opium by isocratic, reversed-phase high-performance liquid chromatography*

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(First received March 4th, 1986; revised manuscript received May 14th, 1986)

Although opium has been in use for centuries, there is no consensus on a uniform method for the simultaneous quantitation of morphine and other related opium alkaloids[1]. The quest for better chromatographic methods for simultaneous separation and quantitation of the principal opium alkaloids such as morphine, codeine, thebaine, papaverine and noscapine still continues, as revealed by a survey of the recent literature. The major problems associated with the analysis of gum opium alkaloids are: (i) complexity of the matrix itself; (ii) incomplete recovery of alkaloids from the matrix during extraction due to solubility limitations and (iii) strong adsorption of polar alkaloids like morphine on column packings.

Some classical methods are known to yield low assay results for morphine in gum opium[2]. Further, pharmacopoeial methods involve a rather tedious preseparation of morphine from the matrix before it is quantitated. The limitations and difficulties of some classical methods for quantitation of opium alkaloids have been briefly reviewed by Ziegler *et al.*[3].

Several chromatographers have observed that underivatized morphine is difficult to quantitate by gas chromatography. This is most probably due to its high polarity which favours strong adsorption at active sites of the column packings, resulting in deceptively low results[4].

Eventually, attention was focused on high-performance liquid chromatography (HPLC). The use of HPLC in straight-phase, reversed-phase, ion-pair and ion-exchange modes for separation of opium alkaloids has been reviewed[4–7]. The reversed-phase mode using bonded cyano[8,9], amino[10], octyl[11], phenyl[12], and octadecyl[8,13,14] packings and the ion-pair mode[13,15–18] have been widely studied for separation of the principal alkaloids of gum opium. However, almost all chromatographers have used either expensive acetonitrile and/or ion-pairing reagents as mobile phase components to achieve simultaneous separation.

We now report a simple, inexpensive, isocratic, reversed-phase HPLC method using a bonded phenyl column for simultaneous separation of the five principal alkaloids of gum opium without using expensive solvents such as acetonitrile or ion-pairing reagents.

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* NCL communication No. 4004.

EXPERIMENTAL

Apparatus

The method was developed on an high-performance liquid chromatographic system (Waters Chromatography Division, Milford, MA, U.S.A.) equipped with two Model 6000A solvent-delivery pumps controlled by a Model 660 solvent programmer, a Model U6K universal injector and a Model 440 absorbance detector (254 nm). The analogue output of the absorbance detector was recorded and processed with a Waters Model 730 data module (printer, plotter and integrator). A Waters μBondapak phenyl stainless-steel column (10 μm, 30 cm × 3.9 mm I.D.) was used.

Chemicals

Sodium acetate and triethylamine were reagent quality. Methanol was purified to chromatographic quality in our laboratory. A Milli-Q system (Millipore Corporation, Bedford, MA, U.S.A.) was used to purify water. Authentic samples of morphine, codeine, thebaine, papaverine and noscapine were obtained from Goverment Opium and Alkaloid Works Undertaking, Neemuch, India.

Mobile phase

A mixture of 42% of 1% (w/v) aqueous sodium acetate containing 7.0 mM triethylamine and 58% methanol was used at a flow-rate of 1.0 ml/min. The pH of the aqueous component of the mobile phase was 11.

Standard mixture of opium alkaloids

About 10 mg each of morphine, codeine, thebaine, noscapine and 3.5 mg papaverine were carefully weighed, dissolved and made up to 100 ml; 25 μl of this solution were injected into the liquid chromatograph.

Extraction of alkaloids from gum opium

A 2-g quantity of gum opium was slurried with 20 ml of 2.5% (v/v) aqueous aceticd acid by vigorous mechanical shaking for 30 min. The slurry was centrifuged and the supernatant layer was decanted and filtered. The extraction procedure was repeated three times.

The extracts were pooled and made up to 100 ml with 2.5% (v/v) aqueous acetic acid. A 5-ml volume of this stock solution was diluted to 100 ml in the mobile phase and 25 μl of the diluted solution were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

We observed that, when the same mobile phase was used, the retention times for opium alkaloids were shorter and the peak shapes more symmetrical on a bonded phenyl phase than those on an octadecyl phase. This prompted us to reason that a simple and cheap aqueous methanolic mobile phase, although rather neglected for alkaloid separation, has sufficient potential for the separation of opium alkaloids. Popl *et al.*[19] recently reported that the most commonly used aqueous methanolic mobile phase has not found wide use in the separation of alkaloids even on the popular octadecyl phase.

During the optimization of the composition of the methanolic aqueous mobile phase for the separation of principal gum opium alkaloids on the phenyl column, we found it advantageous to work at an apparent pH higher than 8, considering the pK_a values (morphine 7.87 and 9.85, codeine 7.95, thebaine 7.95, noscapine 6.18 and papaverine 5.90). Wehrli et al.[20] reported that when it is necessary to work at a high apparent pH (>8), triethylamine is virtually optimal as an organic modifier on the basis of minimum silica attack, retention and separation properties, availability, handling and good solubility. Using a mobile phase composition similar to the one stated in the Experimental section, but adjusting the pH of the aqueous component to 5.5 (instead of 11.0) with glacial acetic acid, inadequate separation was observed. On raising the pH of the aqueous component to 11 with triethylamine (0.1%, v/v in 1%, w/v aqueous sodium acetate) the chromatogram improved dramatically. Morphine was then adequately retained, tailing was drastically reduced and the selectivity factors, α, for alkaloid pairs became favourable. This led to resolution of all the peak pairs under isocratic conditions and within a reasonable time (about 30 min).

In spite of using the optimized mobile phase over a period of 3 months without a guard column, there was apparently no column deterioration. The high percentage (58%, v/v) of methanol appears to have some protective influence on the silica matrix of the packing at the high apparent pH value (pH* 10.5). However, for prolongation of column life, an appropriate guard column is recommended.

Triethylamine has a dual rôle. It masks free residual silanol sites of the packing, which helps to reduce tailing, and also raises the apparent pH value of the mobile phase to about 10.5, which presumably keeps the alkaloids essentially in their non-ionic forms, facilitating their separation. We found that an addition of 1% (w/v) sodium acetate to water helps to achieve adequate retention (k' about 0.5) of morphine, which can be explained by the salting-out effect.

Substitution of expensive acetonitrile for methanol in the optimized mobile phase did not result in any particular advantage. When the methanol was replaced by an equivalent amount of tetrahydrofuran in the optimized mobile phase, morphine was not adequately retained (k' about 0.1).

There appears to be some difference in the respective k' values (4.97 and 5.44 respectively) for thebaine as seen from Figs. 1 and 2. This is because the weight of thebaine in the injected volume of the standard solution was about 4.5 times that present in the same volume of gum opium extract. It is known that k' values tend to decrease with large increases in the amount of solute. Therefore, we injected a fresh solution of an artificial mixture of principal gum opium alkaloids, the concentration of each matching that in the gum opium extract used for injection. We verified that very similar k' values for all the alkaloids were obtained in both cases, which is significant considering that some peaks are eluted close to morphine and codeine.

Using the optimized mobile phase, we achieved a baseline separation of a standard mixture of all the five principal alkaloids within about 30 min at a flow-rate of 1.0 ml/min (Fig. 1). Subsequently, we successfully used this mobile phase for the separation of principal alkaloids in extracts from real Indian gum opium (Fig. 2).

The only previous reference to a simultaneous separation of all the five principal opium alkaloids on a bonded phenyl column is by Pettit and Damon[12] who reported a method in connection with a poppy straw concentrate. However, in spite of using a linear gradient, the separation of the papaverine–noscapine pair was in-
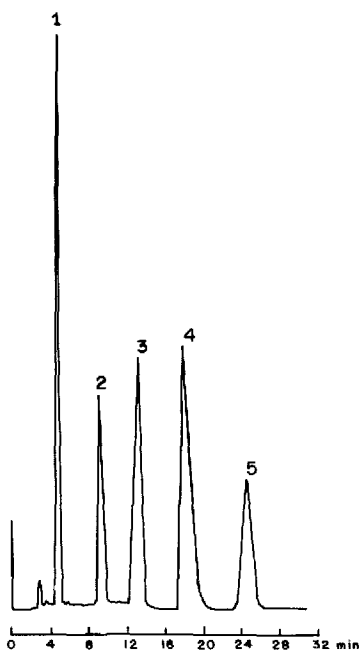
Fig. 1. Separation of an artificial mixture of five principal opium alkaloids. Column: μBondapak phenyl (300 mm × 3.9 mm I.D.). Eluent: water (1%, w/v sodium acetate and 7.0 mM triethylamine–methanol (42:58). Flow-rate: 1.0 ml/min. UV detection at 254 nm. Peaks: 1 = morphine; 2 = codeine; 3 = papaverine; 4 = thebaine; 5 = noscapine.

Fig. 2. Chromatogram of the gum opium extract. Details as in Fig. 1.

adequate, which makes the method less suitable for routine simultaneous analysis of all the five gum opium alkaloids.

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