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Note

Separation and determination of opium alkaloids by high-performance liquid chromatography

YOSHIFUMI NOBUHARA, SHIORI HIRANO, KENSUKE NAMBA and MASAHISA HASHIMOTO

Research Laboratories, Dainippon Pharmaceutical Co., Ltd., 33-94 Enoki-cho, Suita, Osaka 564 (Japan)

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High-performance liquid chromatography (HPLC) was first used for the separation of alkaloids in opium in 1973 by Wu *et al.*[1], who separated four opium alkaloids and heroin. Since then, various chromatographic systems such as ion-exchange[2], adsorption[3–7] and normal and reverse partition chromatography[1,8–10], have been adopted. For the simultaneous separation and determination of five or six major alkaloids in gum opium, procedures have been reported of gradient elution from an adsorption mode column and of isocratic elution in an ion-pair reversed-phase chromatography.

This paper describes a simple and rapid method for the routine quantitative analysis of the six major alkaloids in gum opium by direct isocratic HPLC on a reversed-phase partition mode column, without using ion-pair reagents.

EXPERIMENTAL

Apparatus

A Waters Model ALC/GPC 204 liquid chromatograph, equipped with a Model 6000A pump, a Model 440 detector (254 nm) and a Model U6K injector (Waters Assoc., Milford, Mass., U.S.A.) was used.

The columns were stainless-steel tubes (300 × 4 mm I.D.), packed with Nucleosil 10CN or Nucleosil $10C_{18}$ (Macherey, Nagel & Co., Düren, G.F.R.).

Mobile phase

A mixture of 1% ammonium acetate buffer (pH adjusted with acetic acid) and acetonitrile, or a mixture of the buffer, acetonitrile and dioxane was used.

Alkaloids standard solution

10 mg of morphine, 10 mg of codeine, 10 mg of cryptopine, 10 mg of thebaine, 10 mg of narcotine and 2 mg of papaverine were dissolved in methanol, and made up to 20 ml.

Determination of alkaloids in gum opium

A 2-g amount of gum opium was mechanically shaken with 20 ml of 2.5%

acetic acid for 20 min. Then the mixture was centrifuged, and the supernatant was separated and filtered. The extraction procedure was repeated three times.

The extracts were combined and made up to 100 ml with 2.5% acetic acid. A 5-ml volume of the aqueous acetic solution was diluted to 20 ml with methanol, and 6 μl of the solution was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The retention times of the alkaloids were dependent on the composition of the mobile phase (Table I) and the pH of the buffer (Fig. 1). The six alkaloids were completely separated with both columns in the pH range 5.5–6.0 (Figs. 2 and 3).

TABLE I

RETENTION TIMES OF ALKALOIDS

Systems A–D: column, 300 × 4 mm I.D. Nucleosil 10CN; systems E–G: column, 300 × 4 mm I.D. Nucleosil $10C_{18}$. Mobile phases: A, 1% ammonium acetate (pH 5.8)–acetonitrile–dioxane (80:10:10); B, 1% ammonium acetate (pH 5.8)–acetonitrile (80:20); C and E, 1% ammonium acetate (pH 5.8)–acetonitrile (70:30); D and G, 1% ammonium acetate (pH 5.8)–acetonitrile (60:40); F, 1% ammonium acetate (pH 5.8)–acetonitrile (65:35). All systems: flow-rate, 1.5 ml/min.

Alkaloid	*Retention time (min)*						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Morphine	4.1	4.2	4.0	3.8	2.4	2.3	2.2
Codeine	5.1	5.4	4.8	4.5	3.3	3.0	2.9
Cryptopine	8.1	9.6	7.2	5.6	6.5	4.7	4.1
Thebaine	9.2	10.8	8.0	6.7	9.8	7.5	6.7
Narcotine	12.3	14.1	9.3	5.9	42.6	23.4	15.7
Papaverine	15.7	18.2	9.3	5.6	20.8	11.3	8.0

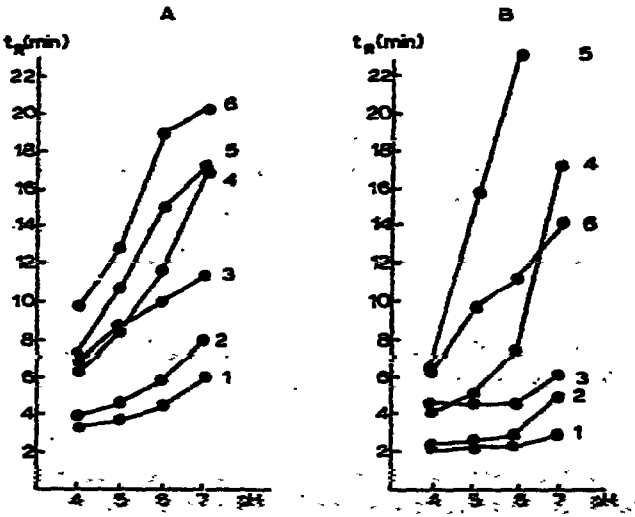

Fig. 1. Effect of pH of the buffer on the retention times (t_R) of alkaloids. (A) Column, Nucleosil 10CN (300 × 4 mm I.D.); mobile phase, 1% ammonium acetate buffer–acetonitrile (80:20); flow-rate, 1.5 ml/min. (B) Column, Nucleosil $10C_{18}$ (300 × 4 mm I.D.); mobile phase, 1% ammonium acetate buffer–acetonitrile (65:35); flow-rate: 1.5 ml/min. 1 = Morphine; 2 = codeine; 3 = cryptopine; 4 = thebaine; 5 = narcotine; 6 = papaverine.

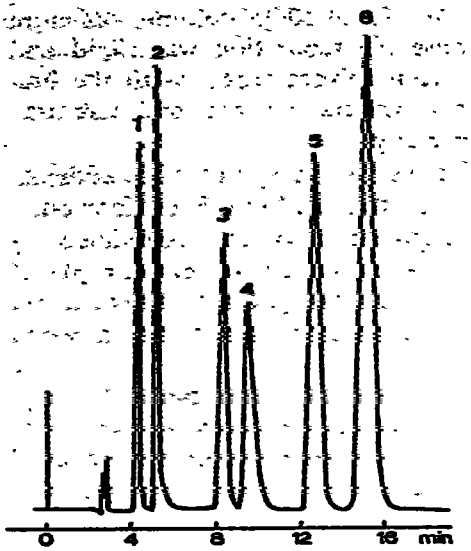

Fig. 2. Chromatogram of six alkaloids on Nucleosil 10CN. Mobile phase, 1% ammonium acetate (pH 5.8)–acetonitrile–dioxane (80:10:10); flow-rate, 1.5 ml/min. 1 = Morphine; 2 = codeine; 3 = cryptopine; 4 = thebaine; 5 = narcotine; 6 = papaverine.

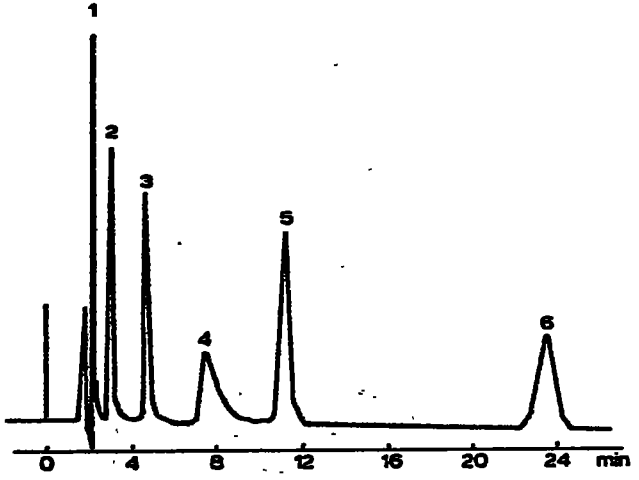

Fig. 3. Chromatogram of six alkaloids on Nucleosil $10C_{18}$. Mobile phase, 1% ammonium acetate (pH 5.8)–acetonitrile (65:35); flow-rate, 1.5 ml/min. 1 = Morphine; 2 = codeine; 3 = cryptopine; 4 = thebaine; 5 = papaverine; 6 = narcotine.

The elution of the alkaloids from the medium-polar column (Nucleosil 10CN) was similar to that from the non-polar column (Nucleosil $10C_{18}$). The cyano-type packing material is known to be either normal-phase or reversed-phase, respectively, when a non-polar or polar mobile phase is used. We used a polar mobile phase to separate the alkaloids by a reversed-phase mechanism.

For the determination of the alkaloids the Nucleosil 10CN column was more suitable than the Nucleosil $10C_{18}$ column, because the separation was rapid and complete and morphine was eluted far enough from solvent front. With the Nucleosil $10C_{18}$ column, morphine was eluted very near the solvent front and narcotine was retained too long to be determined simultaneously.

The conditions for the separation of six alkaloids, namely, morphine, codeine, cryptopine, thebaine, papaverine and narcotine, were studied, but other minor alkaloids are also present in opium. It may be possible to separate simultaneously additional opium alkaloids by adopting a proper composition and optimum pH of the mobile phase. For example, seven alkaloids, adding narceine to above six alkaloids, were successfully separated with the chromatographic conditions, shown in Fig. 4.

Five major alkaloids in gum opium were determined by extraction with 2.5% acetic acid and injection on the medium-polar column, but cryptopine was not detected, since the gum opium in the present study contained an extremely small amount of cryptopine (Fig. 5). The determination was accomplished by preparation

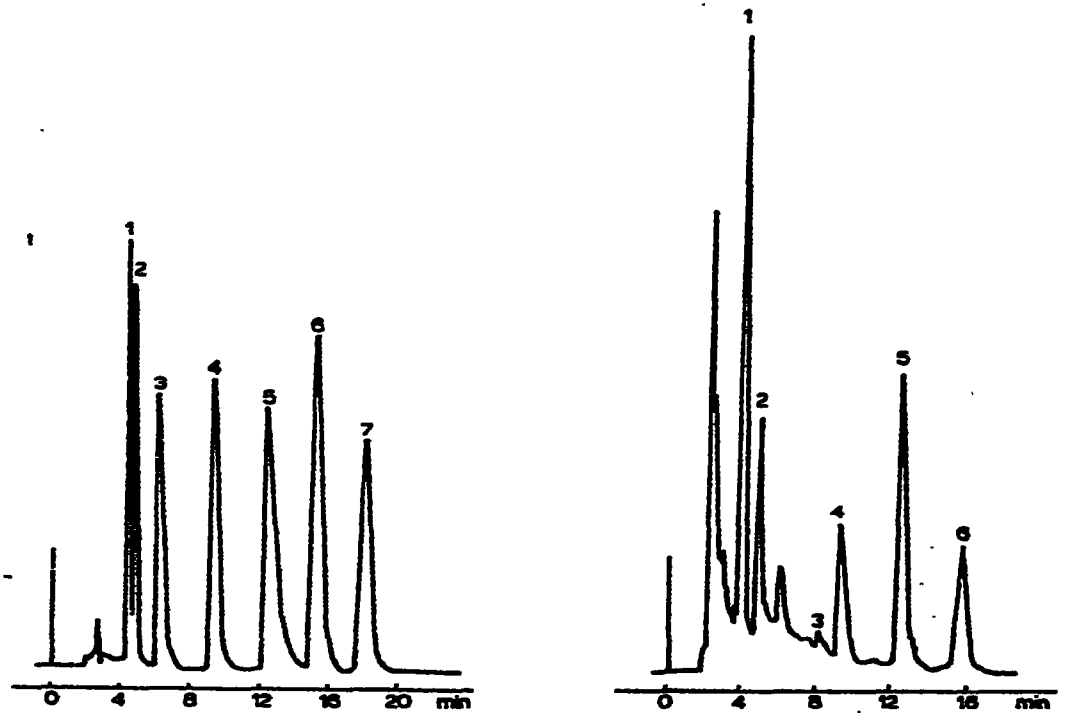

Fig. 4. Chromatogram of seven alkaloids. Column, Nucleosil 10CN (300 × 4 mm I.D.); mobile phase, 1% ammonium acetate (pH 6.3)–acetonitrile–dioxane (79:16:5); flow-rate, 1.5 ml/min. 1 = Narceine; 2 = morphine; 3 = codeine; 4 = cryptopine; 5 = thebaine; 6 = narcotine; 7 = papaverine.

Fig. 5. Chromatogram of the extract from gum opium. Column, Nucleosil 10CN (300 × 4 mm I.D.); mobile phase, 1% ammonium acetate (pH 5.8)–acetonitrile–dioxane (80:10:10); flow-rate, 1.5 ml/min. 1 = Morphine; 2 = codeine; 3 = cryptopine; 4 = thebaine; 5 = narcotine; 6 = papaverine.

of the calibration curve for each alkaloid and measurement of the height of the corresponding peak in the chromatogram of the extract.

The coefficient of variation of the method by repeated analyses for the individual alkaloid was less than 1.5% (Table II).

This method is very convenient for the simultaneous determination of opium alkaloids in routine analysis, as the preparation of sample is easy, the chromatographic system is simple, the analysis time is short and the precision is satisfactory.

TABLE II

REPRODUCIBILITY OF THE DETERMINATION OF ALKALOIDS IN GUM OPIUM

Gum opium from India (No. 77-3, 8). $n = 16$.

	Morphine	*Codeine*	*Thebaine*	*Narcotine*	*Papaverine*
Average	11.52%	3.50%	2.22%	6.64%	0.40%
S.D.	0.163	0.029	0.029	0.094	0.006
C.V.	1.4%	0.8%	1.3%	1.4%	1.5%

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