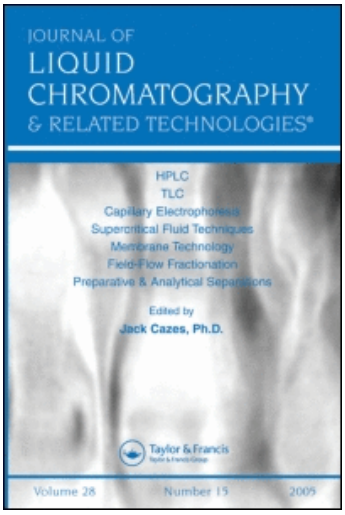

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SIMULTANEOUS DETERMINATION OF IMPORTANT ALKALOIDS IN *PAPAVER SOMNIFERUM* USING REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple and rapid method for the quantitation of eight pharmacologically important drugs, morphine (**1**), codeine (**2**), oripavine (**3**), codeinone (**4**), reticuline (**5**), thebaine (**6**), papaverine (**7**), and narcotine (**8**), in *Papaver somniferum* samples by reversed phase liquid chromatography with photodiode array detection is described. The separation of these compounds was performed with acetonitrile-phosphate buffer (pH maintained to 3.8 using acetic acid) (20 : 80) using a Durasil C_{18} column with 10-μm particles (250 mm × 4.6 mm I.D.).

INTRODUCTION

Papaver somniferum (L.), commonly known in trade as opium poppy, is a well known source of pharmaceutically important alkaloids,[1] mainly morphine, codeine, thebaine, papaverine, and narcotine. Analysis of codeinone, reticuline, and oripavine is of importance due to their role in the biosynthesis[2] of pharmaceutically active alkaloids. Analytical procedures[3] viz. gravimetric, volumetric, colorimetric, spectrofluorimetric, TLC, PC, GLC, and HPLC have been reviewed on the analysis of different opium alkaloids. Analytical reports

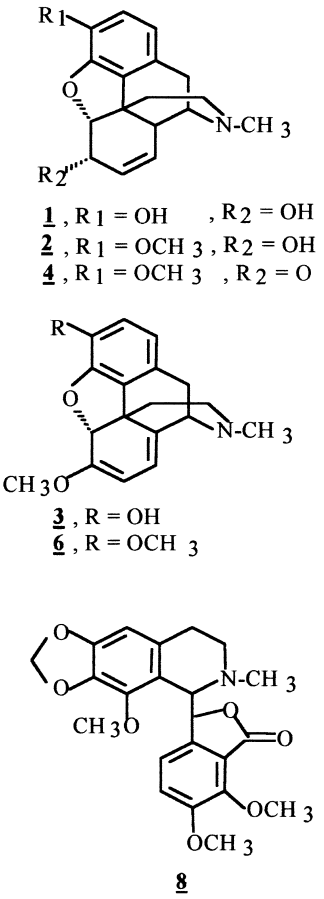

Figure 1. Substances studied.

are mainly on the simultaneous analysis of up to five alkaloids, morphine, codeine, thebaine, papaverine, and narcotine. In our efforts towards developing liquid chromatographic procedures for plant drug analysis (**4**-**8**), here, in this paper we are reporting a reversed-phase HPLC (RPLC) method for the simultaneous analysis of eight major alkaloids (**1**-**8**) (Fig.1) in *P. somniferum* samples by employing a binary mobile phase. This is the first report on the quantitation of eight alkaloids (**1**-**8**) by RPLC.

EXPERIMENTAL

Plant Material and Standards

Compounds **1-5** were purchased from Sigma, USA. Compound **6** was obtained from Prof. H. Rapoport, where as, compounds **7-8** were obtained from Prof. E. Brochmann - Hanssen. Reagents used were HPLC grade (J. T. Baker, USA). Plant material of *P. somniferum* was grown in the experimental farm of this Institute at Lucknow. The sample material of the genotypes used are available in the Gene Bank of this Institute.

Chromatographic Apparatus and Conditions

A Shimadzu (Japan) LC-8A gradient high performance liquid chromatography instrument equipped with two LC-8A pumps, controlled by a CBM-10A interface module, a Model 7725 I manual injector valve (Rheodyne), a 20 μL sample loop, and a SPD-M10AVP (Shimadzu) Photodiode array detector was used for peak purity test and analysis of compounds. Data were collected and analysed using a class LC-10 work station equipped with a Pentium computer (Compaq, Singapore) and a HP Deskjet printer. Solvents were filtered using a Millipore system and analysis was performed on a Merck Durasil C_{18} column (250 mm × 4.6 mm I.D. 10 μm). A constant flow-rate of 1 mL/ min was used during analysis. The composition of mobile phase was optimized by varying the percentage of acetonitrile in phosphate buffer, resulting in the following operating conditions: acetonitrile - 0.1M phosphate buffer-glacial acetic acid (20:80:0.4,v/v/v), pH 3.8; flow rate, 1 mL/min; column temperature, 26°C; detector wavelength, 240 nm, the absorption maxima close to all the compounds.

Sample Preparation

Samples of air dried and powdered capsules (1 g) was extracted with methanol three times (10 mL each time for 3h) and the combined extracts were filtered, concentrated under vacuum, and made up to 1 mL in volume in methanol which was filtered through a Millipore filter. Aliquotes were subjected to HPLC under the above conditions. The contents of each compound (**1-8**) was calculated using an external standard.

Calibration Graphs

Freshly prepared solutions of compounds **1-8** in methanol (1 mg/mL) were used for the preparation of calibration graphs.

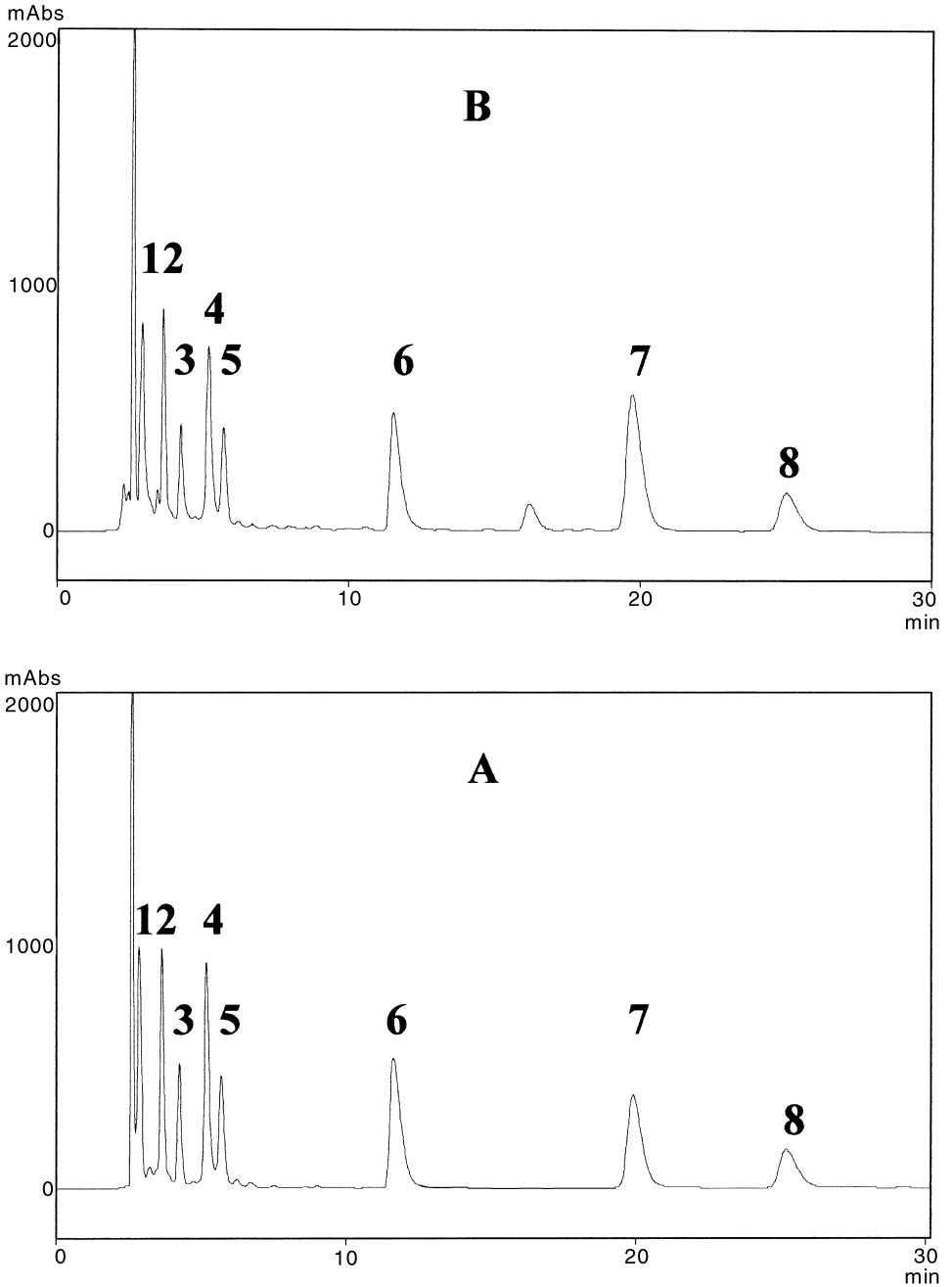

Figure 2. RPLC separation of alkaloids (1-8) in an artificial mixture of pure compounds (A); 1 mg/mL and a *Papaver somniferum* capsule extract (B). Conditions: Durasil C_{18} column; UV detection at 240 nm; mobile phase, acetonitrile -0.1M phosphate buffer-glacial acetic acid (20:80:0.4); flow rate, 1 mL/min. (1) morphine; (2) codeine; (3) oripavine; (4) codeinone; (5) reticuline; (6) thebaine; (7) papaverine; (8) narcotine.

RESULTS AND DISCUSSION

The composition of the mobile phase was optimized using different proportions of acetonitrile in phosphate buffer, the final result being acetonitrile-0.1M phosphate buffer-glacial acetic acid (20:80:0.4, v/v/v), pH 3.8. Figure 2 illustrates the separation of the compounds **1**-**8** in an artificial mixture of standards and a plant sample extract. Peaks corresponding to compounds **1**-**8**, checked via addition of standards, were symmetrical. The peak purity of compounds **1**-**8** was tested using a photodiode array detector: compound **1**, purity up 0.9991, down 0.9974; compound **2**, purity up 0.9977, down 0.9996; compound **3**, purity up 0.9990, down 0.9995; compound **4**, purity up 0.9978, down 0.9991; compound **5**, purity up 0.9995, down 0.9996; compound **6**, purity up 0.9997, down 0.9981; compound **7**, purity up 0.9999, down 1.0000; compound **8**, purity up 0.9998, down 0.9999. The similarity of compounds **1**-**8** in the sample and standards was also checked and found to be 0.9960, 0.9999, 0.9993, 0.9980, 0.9996, 0.9949, 0.9995, and 0.9950 for compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **8**, respectively. Peak purity test results and similarity of compounds **1**-**8** were satisfactory. Recoveries of compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **8** were 96, 96, 97, 96, 96, 97, 96, 96%, respectively. For the examination of recovery, known amounts of freshly prepared solutions of pure compounds **1**-**8** were added to the poppy capsule extract and the quantitation was repeated four times.

Linearity was determined by using five concentrations in a working range of 1-20 μg of each component **1**-**8**. Linear regression equations and correlation coefficients (r) for all the compounds **1**-**8** have been presented in Table 1.

Table 1

Column Performance and Linear Regression Data for Opium Alkaloids*

Compound	Rt	Recovery (%) ± S.D.	Capacity Factor	No. of Theoretical Plate Counts	Linear Regression Equation[a]	r
1	2.90	96 ± 2	0.27	2057	Y = 2660.9X + 64.2	0.999
2	3.63	96 ± 3	0.60	4954	Y = 2616.3X - 57.9	1.000
3	4.23	97 ± 2	0.86	5037	Y = 1414.5X + 29.0	1.000
4	5.18	96 ± 2	1.28	4571	Y = 2769.8X + 46.0	0.999
5	5.70	96 ± 2	1.50	5994	Y = 1544.8X - 16.0	1.000
6	11.53	97 ± 2	4.07	4674	Y = 4762.1X - 43.8	1000
7	19.72	96 ± 3	7.67	6645	Y = 4584.4X - 74.7	0.999
8	25.01	96 ± 3	9.99	8010	Y = 2293.6X - 49.7	1.000

* (1- 8). [a] Number of data points, 5; number of replicates, 3.

Table 2

Percent Content of Specific Alkaloids (1-8) in the Capsules of *Papaver Somniferum* Accessions

S. No.	Accession	% Content of [a] Morphine	Codeine	Oripavine	Codeinone	Reticuline	Thebaine	Papaverine	Narcotine
1	PC 1	0.245 ± 0.004	0.095 ± 0.002	<0.001	0.045 ± 0.002	0.079 ± 0.008	0.012 ± 0.003	0.107 ± 0.001	0.050 ± 0.030
2	PC 2	0.486 ± 0.088	0.061 ± 0.003	<0.001	<0.001	0.307 ± 0.009	0.135 ± 0.002	0.072 ± 0.005	0.469 ± 0.006
3	PC 3	0.114 ± 0.005	0.011 ± 0.004	<0.001	<0.001	0.003 ±0.002	0.016 ± 0.002	<0.001	<0.001
4	PC 4	0.275 ± 0.041	0.052 ± 0.005	<0.001	<0.001	0.016 ± 0.007	0.009 ± 0.002	0.048 ± 0.009	<0.001
5	PC 5	0.399 ± 0.012	0.102 ± 0.017	0.104 ± 0.007	<0.001	<0.001	0.024 ± 0.003	0.078 ± 0.009	0.085 ± 0.006

[a] = Mean values, based on 3 observations, along with standard deviations are reported.

Calibration plots of peak areas versus concentrations were linear, with r values between 0.999 - 1.000. These values indicated good linearity in the examined concentration range.

Detection limit was determined by estimating the minimal mass of each compound (**1**-**8**) that can be quantified. The values were in between 0.1 to 0.2 μg/injection for compounds **1**-**8**.

The method reported here was applied for the analysis of a number of *P. somniferum* accessions for all the eight compounds (**1**-**8**) and results of a few have been presented in Table 2.

CONCLUSION

The described method allows a simple, rapid, and simultaneous separation of eight important alkaloids present in *P. somniferum* capsule husk. The method is suitable for the rapid screening for the quality of alkaloids present in the opium of *P. somniferum* accessions. Also, the quantitation of intermediates in the biosynthetic pathway of pharmacologically active alkaloids may help in the molecular disection of the concerned pathway.

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