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## Note

# Analysis of poppy straw concentrate by high-performance liquid chromatography

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Poppy straw concentrate, an extract of the dried pods and stems of *Papaver somniferum*, was first imported in 1975. At that time there was concern that the supply of opium might not be sufficient to satisfy the needs of our pharmaceutical industry for morphine. While free of duty, these importations must still be analyzed by Customs' laboratories as statistics are kept on the amounts, expressed as anhydrous morphine, being imported.

A number of high-performance liquid chromatographic (HPLC) methods for the analysis of opium and opium alkaloids in pharmaceuticals have been published[1–12]. From these it was decided that an HPLC method held the most promise for a rapid and accurate analysis, especially if it could be combined with a simplified sample preparation.

This report presents a reversed-phase HPLC method for the analysis of the five major alkaloids in poppy straw concentrate, using a phenyl type bonded phase and employing a simple and rapid sample preparation step.

## EXPERIMENTAL

### *Chemicals*

The acetonitrile and water were both HPLC grade. Alkaloid standards, U.S.P. grade, were obtained from Merck (Rahway, NJ, U.S.A.) and were recrystallized before use. The internal standard, quinine sulfate, was J. T. Baker (Phillipsburgh, NJ, U.S.A.) ULTREX grade. Dimethyloctylamine was obtained from Alfa Products (Dovers, ME, U.S.A.). All other chemicals were reagent grade. Poppy straw samples were from importations.

### *Apparatus*

The liquid chromatograph was a Waters Associates Model 201, equipped with two 6000A pumps, a Model 660 solvent programmer, a U6K injector and a Perkin-Elmer (Coleman) Model LC-55 variable wavelength detector. A Perkin-Elmer Model 1 computing integrator provided areas and retention times. The column was a phenyl Bondapak (Waters), 25 cm × 5 mm I.D. and was used in conjunction with a 7 cm × 2 mm I.D. guard column packed with $C_{18}$/Corasil (Waters), particle size 37–50 μm.

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*Procedure*

Approximately 50 mg of sample were accurately weighed into a small erlenmeyer flask. To this were added 25 ml of solvent A containing 1 mg/ml of the internal standard. Solution was effected by sonication for 30 min. An aliquot of this solution was filtered through a Gelman (Ann Arbor, MI, U.S.A.) Acrodisc-CR filter, pore size 0.45 $\mu$m. Standards were dissolved in the same batch of solvent plus internal standard as the sample, and treated in the same manner. Injections were of 10 $\mu$l.

A linear gradient of 20 min duration was employed. Solvent A was acetonitrile–water (5:95) and solvent B was acetonitrile–water (20:80). Both solvents contained 1 ml/l of glacial acetic acid and 0.04 ml/l of N,N-dimethyloctylamine. The pH of both was adjusted to 3.5 with sodium hydroxide. Degassing was accomplished by sonication under vacuum. The column was allowed to re-equilibrate for 10 min between samples. The flow-rate was 1.0 ml/min. The column eluate was monitored at 275 nm, which reduced the contribution from interfering peaks. Standards were run at the start, in the middle and at the end of a sample series.

## RESULTS AND DISCUSSION

Representative chromatograms of a standard (B) and sample (A) are shown in Fig. 1. The choice of quinine sulfate as the internal standard was dictated by its retention time which placed it in a portion of the chromatogram free of interfering

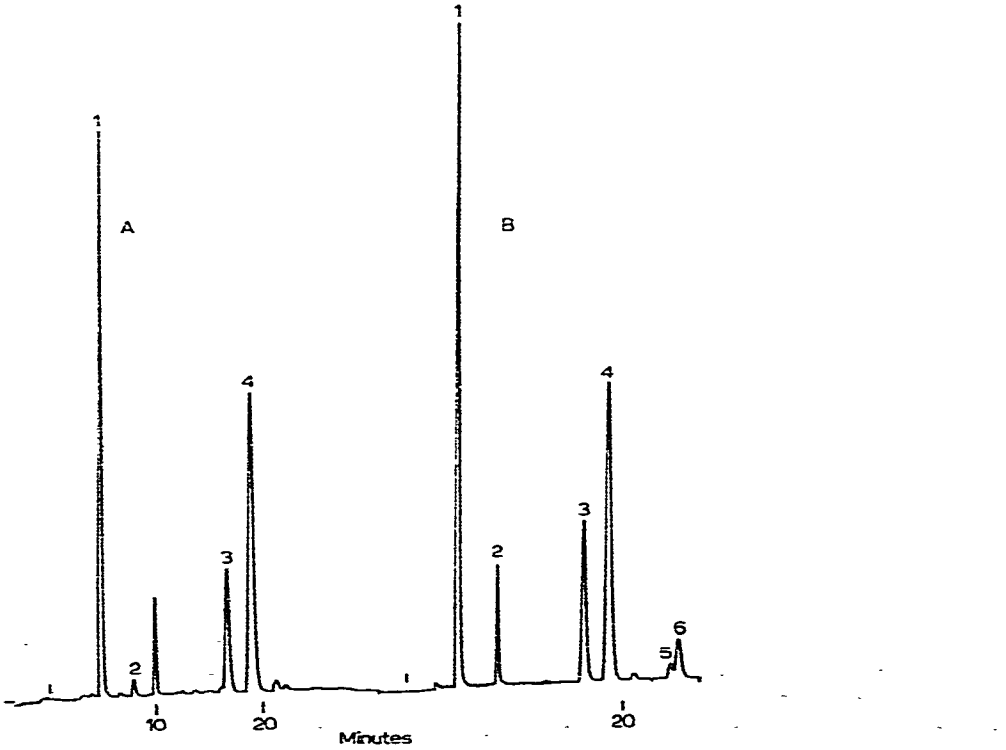


Fig. 1. Chromatograms of sample (A) and standard (B). Peaks: 1 = Morphine; 2 = codeine; 3 = thebaine; 4 = quinine; 5 = papaverine; 6 = narcotine.

TABLE I

PERCENT ANHYDROUS ALKALOID

| *Country of origin* | | *Sample* | *Morphine* | *Codeine* | *Thebaine* |
|---|---|---|---|---|---|
| Holland | A | 1 | 74.6 | | |
| | B | 2 | 69.0 | | |
| | | 3 | 72.1 | | |
| | | 4 | 71.9 | | |
| | C | 5 | 70.7 | | |
| | | 5a* | 70.3 | | |
| | | 6 | 76.6 | | |
| | | 6a* | 77.4 | | |
| | | 7 | 74.4 | | |
| France | A | 8 | 63.4 | 2.7 | 1.6 |
| | B | 9 | 57.0 | 2.0 | 4.1 |
| | | 10 | 58.3 | 1.9 | 3.8 |
| | | 11 | 58.4 | 1.9 | 3.8 |
| | C | 12 | 59.5 | 2.0 | 3.9 |
| | | 13 | 59.6 | 1.9 | 4.1 |
| | | 13a* | 60.0 | 1.8 | 4.0 |
| | | 14 | 58.5 | 1.9 | 3.4 |
| Poland | | 15 | 65.1 | | |
| Yugoslavia | | 16** | 78.1 | 2.0 | 1.0 |

* Replicate

** Also contained 1.0% narcotine.

peaks. The phenyl column gave superior peak shapes compared to either an octyldecyl or octyl type; however, the presence of N,N-dimethyloctylamine was found to be essential in eliminating tailing.

Sixteen samples of poppy straw concentrate were analyzed. These represented eight separate lots from four countries. As a check on precision, three samples (5, 6 and 13) were run in duplicate and parallel. The results are shown in Table I. Repeat injections (four) of sample 9 gave a value for the morphine concentration of 57.0 $\pm$ 0.4% with a coefficient of variation of 0.6%. As the data in Table I shows, the reproducibility as evidenced by the replicates and within lot analyses is satisfactory,

TABLE II

LINEARITIES AND DETECTION LIMITS

| *Alkaloid* | *Linear range (%)* | *Detection limit (%)* |
|---|---|---|
| Morphine | 10–100 | – |
| Codeine | 0.3–10 | 0.1 |
| Thebaine | 1.0–6.0 | 0.5 |
| Papaverine | 0.5–5.0 | 0.1 |
| Narcotine | 0.5–5.0 | 0.2 |

with one exception. This exception is lot C from Holland, samples 5–7. This lot was subsequently analyzed by both a gas chromatographic and polarographic method[13]. Both analysis confirmed the HPLC results. Thus it is probable that the observed values are due to a sampling problem. Previous work within the laboratory system had shown that the concentrations of alkaloids in poppy straw concentrate varied over only a limited range. Thus the linearity of detector response was investigated only over these expected ranges. These results are shown in Table II. This table also contains the practical minimum detection limits for the minor alkaloids. A practical limit of detection was considered to be three times the noise level.

The method presented is simple, reproducible and sensitive. Eleven runs (three standards, eight samples) can be made in a normal day, and sample preparation is easily achieved during the 35 min run time.

## REFERENCES


1 J. D. Wittwer, *J. Forensic Sci. Soc.*, 18 (1973) 138–142.
2 S. E. Hays, L. T. Grady and A. V. Kruegel, *J. Pharm. Sci.*, 62 (1973) 1509–1513.
3 T. H. Beasley, D. W. Smith, H. W. Zeigler and R. L. Charles, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 85–90.
4 P. J. Twitchett and A. C. Moffat, *J. Chromatogr.*, 111 (1975) 149–157.
5 H. W. Zeigler, T. H. Beasley and D. W. Smith, *J. Ass. Offic. Anal. Chem.*, 58 (1975) 888–897.
6 V. Das Gupta, *J. Pharm. Sci.*, 65 (1976) 1697–1698.
7 I. Lurie, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 1035–1040.
8 C. Y. Wu and J. J. Wittick, *Anal. Chem.*, 49 (1977) 359–363.
9 B. B. Wheals and I. Jane, *Analyst (London)*, 102 (1977) 625.
10 P. C. Vincent and B. F. Engelke, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 310–314.
11 G. K. Poochikian and J. C. Cradock, *J. Chromatogr.*, 171 (1979) 371–376.
12 E. J. Kubiak and J. W. Munson, *J. Pharm. Sci.*, 69 (1980) 152–156.
13 R. S. Schwartz and C. R. Benjamin, *8th Annual Meeting, Federation of Analytical Chemistry and Spectroscopy Society, Philadelphia, PA, September 20–25, 1981*, Abstract No. 81.