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Liquid Chromatographic Determination of Morphine in Opium

Opium is obtained from the unripe seed capsules of the opium poppy, *Papaver somniferum*. It contains 25 to 30 alkaloids. Commercially, morphine, narcotine (noscapine), papaverine, thebaine, codeine, and narceine are considered the most important of these alkaloids.

As a result of widespread drug abuse and efforts of law enforcement agencies to enforce drug laws, forensic laboratories presently have large work loads. It is thus necessary that the forensic chemist have at his disposal methods of analysis that are reliable, accurate, fast, and recognized as acceptable by experts in the field.

This paper presents a fast method for determining morphine in opium using high speed liquid chromatography (LC). Retention data for other drugs of forensic interest are also included.

Experimental

Apparatus

A Dupont Model 820 Liquid Chromatograph equipped with 3000 psi pump, 254 mμ ultraviolet detector, Honeywell Electronik recorder, and gradient elution accessory.

Column

A stainless steel column, 1 m $\times$ 2.1 mm inside diameter, packed with DuPont S.A.X. ion exchange material. (S.A.X. is a quaternary ammonium substituted methacrylate polymer coated 1 percent by weight on Zipax).

Reagents

A 0.01 *M* boric acid buffer adjusted to pH 9.5 with 1 *N* NaOH and a 0.01 *M* KH_2PO_4 buffer adjusted to pH 6.0 with 1 *N* NaOH were prepared.

Reference Standards

Morphine sulfate, thebaine, noscapine, papaverine hydrochloride, codeine phosphate, methadone hydrochloride, and iso-methadone hydrochloride were obtained from Mallinckrodt Chemical Works. Cocaine hydrochloride, procaine hydrochloride, quinine hydrochloride, quinidine sulfate, Dionin (ethylmorphine hydrochloride), anileridine, and

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apomorphine hydrochloride were obtained from Merck and Company. Cryptopine and laudanosine were obtained from Pfaltz and Bauer. Methapyrilene hydrochloride was obtained from Sigma Chemical Co. Oxymorphone hydrochloride was obtained from Endo Laboratories. Dihydrocodeinone bitartrate was obtained from The Merrell Company. Demerol hydrochloride (meperidine hydrochloride) was obtained from Winthrop Chemical Company.

All reference standards were dissolved in dimethyl sulfoxide (DMSO). (WARNING—Dimethyl sulfoxide is readily absorbed through the skin so care must be taken in its use.) The concentration of the reference standards used for obtaining retention data was 1 mg/ml. A reference synthetic opium solution was also prepared containing the following quantities of alkaloids in 10.0 ml DMSO:

(a) Morphine sulfate	134.2 mg =	100.92 mg morphine
(b) Codeine phosphate	43.9 mg =	30.95 mg codeine
(c) Thebaine	31.3 mg	
(d) Papaverine hydrochloride	23.5 mg =	23.5 mg papaverine
(e) Noscapine	101.0 mg	

This mix corresponds to the relative amounts of alkaloids found in opium. One ml of this solution was diluted to 10.0 ml for the working standard.

Procedure

Samples of opium were powdered in a mortar and 100–150 mg of each sample were triturated in a mortar with 1 ml DMSO for about 5 min. The DMSO solution was transferred to a 10 ml volumetric flask using a disposable pipet with a rubber bulb. The mortar was rinsed with three 2 ml portions of DMSO and added to the DMSO in the 10 ml volumetric flask and diluted to 10.0 ml with DMSO. Injections of 5.0 μl of each solution were made onto the S.A.X. column.

Instrumental Conditions

Sensitivity: 0.16 OD
Pressure: 1500 psi
Flow: about 0.75 ml/min
Temperature: Ambient
Solvent: Reservoir A: boric acid buffer; Reservoir B: phosphate buffer
Instrument set at gradient – operate position
Initial composition 15 percent B – 85 percent A
Final composition: 100 percent B – 0 percent A

Linear gradient runs were made from 15 percent to 100 percent at 5 percent or 10 percent per minute and held at the final composition. Immediately upon injection of the sample the gradient is started, and the system is allowed to hold the final composition until all of the alkaloids are eluted, which under the above conditions takes about 30 minutes. The instrument is then manually reset to the initial composition of 15 percent B and 85 percent A. A 20–25 minute equilibration with solvent flow is necessary before the next injection is made.

Results and Discussion

The chromatogram of the reference synthetic opium mix is shown in Fig. 1. The separation of the major opium alkaloids is satisfactory. The Thailand opium sample, Fig. 2, is

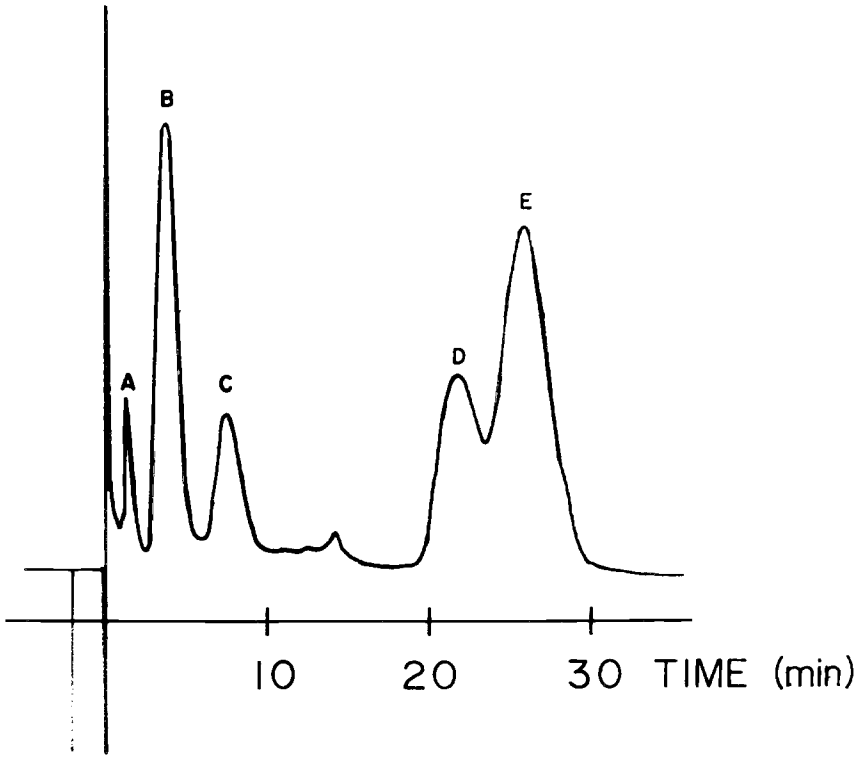

FIG. 1—*Chromatogram of the reference synthetic opium mix. The order of elution is: A = Codeine, B = Morphine, C = Thebaine, D = Noscapine, and E = Papaverine.*

interesting in that it contains a small amount of noscapine, almost no papaverine, and a large amount of morphine.

The morphine content of three opium samples was determined by LC and by the procedure in the AOAC II [*1*]. Quantitations using the LC procedure were made using the area under the curve as determined by triangulation. A comparison of the results of these two procecures is shown in Table 1. The LC procedure gave lower results than the AOAC procedure on the Iranian gum opium and the Thailand opium samples. These results are, however, reproducible from run to run.

Methods of analysis utilizing extraction and separation of components before quantitation are totally dependent on the efficiency of the separation and the completeness of

TABLE 1—*Results of analysis.*

	Morphine, %		
Source of Sample	AOAC	LC	Difference, %
USP Powdered Opium	10.98	10.95	...
Iranian Gum Opium	11.38	9.95	12.6
Thailand Opium	20.41	18.34	10.0

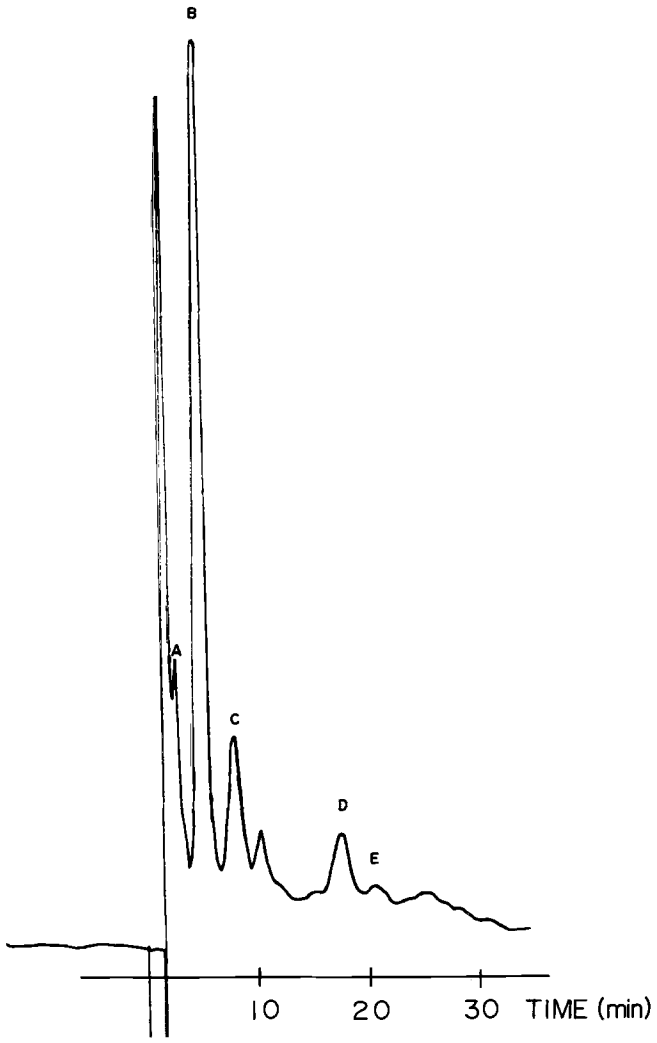

FIG. 2—*Chromatogram of Thailand opium.*

extraction. Smith [2] reported DMSO to be an excellent solvent for opium. The use of DMSO to dissolve the opium precludes an extraction step, and thus one possible source of error is eliminated.

Tables 2 and 3 list retention data for the drugs chromatographed using linear elution with solvent composed of 85 percent boric acid buffer and 15 percent phosphate buffer and by the gradient elution previously described. All retention volumes are expressed relative to methapyrilene. Comparison of Tables 2 and 3 shows the order of elution of noscapine and papaverine is reversed.

The proposed LC assay procedure gives rapid, reproducible quantitation of morphine as well as an identification of codeine, thebaine, noscapine, and papaverine.

TABLE 2—*Linear elution at 15 percent pH 6.0 + 85 pH 9.5 buffer. Retention data is expressed relative to Methapyrilene (retention volume of Methapyrilene = 7.28 ml).*

Methapyrilene	1.00
Morphine	0.51
Thebaine	0.82
Noscapine	3.34
Papaverine	2.82
Codeine	0.29
Cryptopine	0.32
Laudanosine	0.55
Cocaine	0.65
Procaine	0.47
Methadone	2.07
Iso-Methadone	6.39
Quinine	2.75
Quinidine	1.86
Heroin	0.35
Dionin	0.37
Oxymorphone	0.56
Dilaudid	0.51
Dihydrocodeinone	0.39
Anileridine	6.39
Demerol	0.47
Apomorphine	Retained

TABLE 3—*Retention data using pH gradient 15 percent pH 6.0 buffer + 85 percent pH 9.5 buffer to 100 percent pH 6.0 buffer at 5 percent/min. Retention data expressed relative to Methapyrilene (retention time of Methapyrilene = 9.84 min).*

Methapyrilene	1.00
Morphine	0.60
Thebaine	0.88
Noscapine	2.33
Papaverine	2.69
Codeine	0.35
Cryptopine	0.39
Laudanosine	0.65
Cocaine	0.79
Procaine	0.58
Methadone	1.15
Iso Methadone	1.38
Quinine	1.46
Quinidine	1.40
Heroin	0.42
Dionin	0.46
Oxymorphone	0.65
Dilaudid	0.63
Dihydrocodeinone	0.49
Anileridine	1.59
Demerol	0.61
Apomorphine	2.28

Acknowledgement

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References

[1] *The Association of Official Analytical Chemists*, Vol. 2, Chapter 36, section .031, 1970, p. 624.
[2] Smith, E., *Journal of the Association of Official Analytical Chemists*, Vol. 53, 1970, pp. 603–608.