Journal of Chromatography, 294 (1984) 297–302
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,750

ANALYSIS OF ALKALOIDS IN OPIUM

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(First received January 19th, 1984; revised manuscript received March 16th, 1984)

SUMMARY

A rapid analytical procedure for the separation and quantification of the major alkaloids of opium is reported. Separation was effected by Celite column chromatography. Codeine, thebaine, papaverine, and noscapine were eluted in one fraction with an ether–light petroleum (b.p. 30–60°C) mixture while morphine was eluted in a second fraction with a chloroform–diethylamine mixture. Quantification was performed by isothermal gas chromatography. The group of four alkaloids (fraction I) was analyzed directly on a 3% OV-1 column, while the morphine (fraction II) required derivatization with N,O-bis(trimethylsilyl)acetamide prior to its analysis on a 3% OV-1 column. Good reproducibility and linearity were obtained.

INTRODUCTION

The quantification of the alkaloids in opium has always presented problems. In previously reported methods of analysis[1–9], the separation of these alkaloids usually required relatively long and sometimes complex procedures. Ion exchange, overnight soaking, shakeouts, and liquid–liquid extractions have been used in attempts to isolate the alkaloids from the opium gum. These separations have been followed by either high-performance liquid chromatographic (HPLC)[1–6] or gas chromatographic (GC) determinations[7–9]. HPLC methods for opium can be very easily affected by impurities, giving rise to resolution problems and possible invalid quantifications, thus requiring a clean-up procedure. The GC methods generally required temperature programming and attempts to obtain all five alkaloids in a single GC run have not given very satisfactory results for quantitative analysis. It had been our experience that morphine must be derivatized in order to obtain reproducible results by GC analysis.

The method reported utilizes a relatively quick Celite column chromatographic separation followed by isothermal GC for the analytical determination. Codeine, thebaine, papaverine, and noscapine were analyzed together as one fraction. Since the morphine was to be derivatized, it was collected and analyzed separately.

The opium was dried at room temperature prior to analysis, not only to obtain reproducible results, but also to minimize degradation of the alkaloids.

0021-9673/84/$03.00

MATERIALS AND METHODS

Celite 545 (purified siliceous earth), acid washed, was obtained from Johns-Manville, Philadelphia, PA, U.S.A. N,O-bis(trimethylsilyl)acetamide (BSA) was obtained from Pierce, Rockford, IL, U.S.A. Triacontane and docosane were obtained from Applied Science, State College, PA, U.S.A. Diethyl ether, peroxide free, was obtained from Mallinckrodt, Paris, KY, U.S.A. All other solvents were reagent grade chemicals. Column chromatography utilized glass columns 250 mm × 25 mm from Kontes, Vineland, NJ, U.S.A. Morphine, codeine, and papaverine were obtained from S. B. Penick, Lyndhurst, NJ, U.S.A. Thebaine and noscapine were obtained from Mallinckrodt, St. Louis, MO, U.S.A.

Preparation of standard solutions

The triacontane internal standard solution contained 0.65 mg/ml triacontane in chloroform–methanol (9:1). The docosane internal standard solution contained 1 mg/ml docosane in chloroform. The fraction I mixed standard was prepared as follows: approximately 7 mg each of coedeine, thebaine, and papaverine and 20 mg of noscapine were accurately weighed into a 10-ml volumetric flask and brought to volume with the triacontane solution. The morphine standard was prepared by weighing accurately, approximately 2 mg of morphine into a 2-ml glass stoppered tube, adding 0.5 ml of BSA, stoppering the tube and heating for 1 h at 70°C in a heating block. After cooling, 1.0 ml of the docosane interal standard solution was added.

Gas chromatography

A Perkin-Elmer 3920 gas chromatograph equipped with a flame ionization detector and silanized glass columns 6 ft. × 4 mm I.D. packed with 3% OV-1 on Gas-Chrom Q, 100–120 mesh (Supelco, Bellefonte, PA, U.S.A.) was used. The nitrogen flow-rate was 70 ml/min and column oven temperature was about 240°C, but was adjusted to give approximately the following retention times (min): codeine, 3.41; thebaine, 4.66; papaverine, 9.08; triacontane, 15.83; noscapine, 21.33.

The temperature for the morphine analysis was about 233°C, but was adjusted to give approximately the following retention times (min): docosane, 2.50; morphine, 5.83.

Peak areas and calculations were performed on a Spectra Physics SP-4000 data system (Spectra-Physics, Santa Clara, CA, U.S.A.).

Preparation of the opium sample stock solution

The opium was dried at ambient temperature by placing it in a vacuum desiccator over anhydrous calcium sulfate. As the opium dried, it was broken up and ground in a mortar. This process was continued until the opium was dry and reduced to a powder. It was then stored in a desiccator until used.

A 10-g amount of powdered opium was dissolved in 90 ml of dimethyl sulfoxide (DMSO) with gentle heating on a steam bath. The solution was transferred to a 100 ml volumetric flask, cooled, and brought to volume with DMSO.

Column chromatographic separation

A pledget of glass wool was placed in the bottom of a glass chromatographic column. Celite (6 g) which had been thoroughly mixed with 4 ml of 0.5 *N* sodium carbonate was placed in the column and tamped down. The sample layer was then placed on top of this trap layer and tamped down. The sample layer was prepared as follows: 3.0 ml of the opium stock solution was allowed to drain for about 20 min into a beaker. A 1-ml volume of 2 *N* sodium carbonate was added followed by 5 g of Celite. The wet Celite was thoroughly mixed and added to the column as described above. The column was eluted with a water-saturated mixture of ether–light petroleum (7:3) until 240 ml of elute had been collected. This solution was evaporated to a very small volume on a steam bath. It was removed from the steam bath and dried at ambient temperature. This fraction contained codeine, thebaine, papaverine and noscapine (fraction I).

The Celite column was then eluted with water-saturated chloroform to which 0.1% diethylamine had been added. A volume of 225 ml of eluate was collected, and this solution was evaporated to dryness on a steam bath. This fraction contained the morpine (fraction II).

Quantitative analysis

Exactly 10.0 ml of the triacontane internal standard solution was added to fraction I. The alkaloids were analyzed by GC as described. Prior to analysis, an injection of the standard solution was made to saturate the column.

The morphine fraction was dissolved in a chloroform–methanol (3:1) solution, transferred and made to volume in a 25-ml volumetric flask. A 1.0-ml aliquot was transferred to a 2-ml glass-stoppered test tube. The tube was placed in a heating block at 70°C and the solvent evaporated. BSA (0.5 ml) was added to the residue. The tube was stoppered and heated for 1 h at 70°C. After cooling 1.0 ml of the docosane internal standard solution was added. The morphine was then analyzed by GC as previously described.

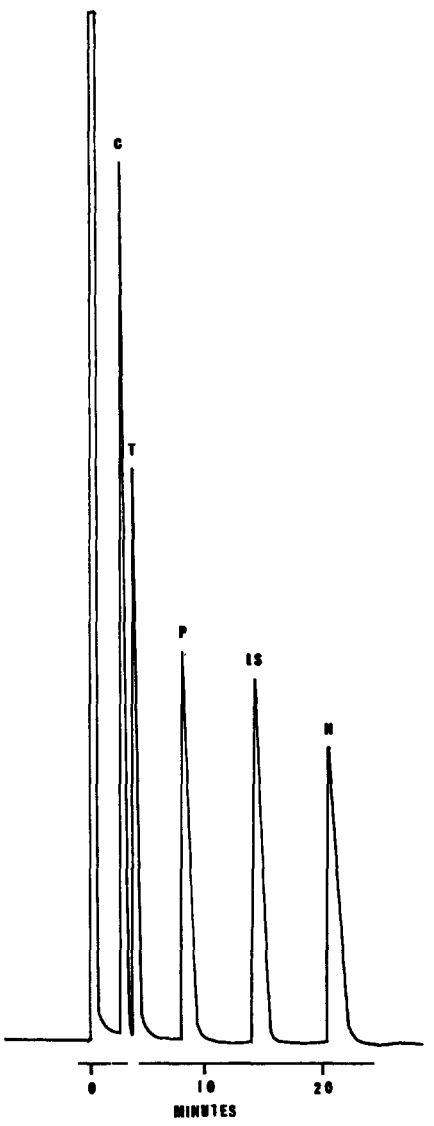
Since it is well known that opium contains many components, both alkaloid fractions were examined to insure that there were no other substances co-eluting or interfering with the alkaloids of interest in the gas chromatogram. Thin-layer chromatographic analysis of the diethyl ether–light petroleum eluate using a toluene–ethyl acetate–diethylamine (70:20:10) system indicated that most of the other chromatographable components of opium were retained on the Celite column. This alkaloid fraction was also examined by GC–MS. No other substances were observed in the alkaloid peaks. As an additional test, the diethyl ether–light petroleum eluate containing the alkaloids was then passed through an 0.5 *N* sulfuric acid–Celite (4 ml/5 g) column. The alkaloids were trapped on this column and the diethyl ether–light petroleum was collected, concentrated on a steam bath and injected in the gas chromatograph under the same conditions as the quantitative determination. There were no peaks having the same retention time as any of the alkaloids of interest.

The morphine fraction was treated in the same manner as above, with similar results, except that the chloroform eluate containing the morphine was passed through a 0.5 *N* sodium hydroxide–Celite (4 ml/6 g) column thereby trapping the morphine. The chloroform was collected and treated under the same conditions as in the quantitative determination of morphine. No peaks were detected in the gas

TABLE I

PERCENTAGE OF ALKALOIDS AND RELATIVE STANDARD DEVIATIONS

Sample	*Morphine*		*Codeine*		*Thebaine*		*Papaverine*		*Noscapine*	
	Percentage	*R.S.D. (%)*	*Percentage*	*R.S.D. (%)*	*Percentage*	*R.S.D. (%)*	*Percentage*	*R.S.D. (%)*	*Percentage*	*R.S.D. (%)*
1	15.0	1.0	4.15	1.5	1.95	1.7	2.22	1.1	9.44	2.7
2	11.7	1.4	1.75	1.1	4.74	0.9	1.4	3.5	4.84	3.0
3	10.4	1.9	4.89	2.8	6.73	4.7	3.15	2.3	6.74	3.6
4	16.1	1.8	2.53	2.3	3.74	3.3	–	–	5.74	2.5
5	14.9	1.2	2.17	4.7	5.03	5.5	–	–	5.55	2.2
6	13.8	3.4	4.10	2.3	1.65	2.5	2.18	1.1	10.6	2.7
7	15.3	0.6	2.58	2.6	2.86	2.7	0.52	4.2	7.32	1.3
8	15.1	2.6	2.21	1.4	2.91	1.7	0.50	1.8	7.27	1.4



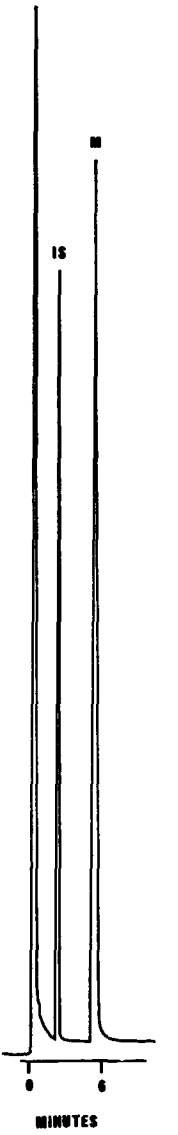


Fig. 1. Chromatogram of opium alkaloids. C = codeine, T = thebaine, P = papaverine, IS = internal standard (triacontane), N = noscapine.

Fig. 2. Chromatogram of morphine analysis. IS = internal standard (docosane), M = morphine.

chromatogram. The morphine fraction was also analyzed by GC–MS and there were no other substances detected in the morphine peak.

RESULTS AND DISCUSSION

A number of samples were analyzed in replicate and relative standard devia-

tions were calculated. The results are shown in Table I. Excellent reproducibility was obtained. Figs. 1 and 2 show typical chromatograms.

Linearity studies showed that codeine and thebaine showed linear behaviour from 0.5 μg to 7 μg, papaverine from 0.9 μg to 7 μg, and noscapine from 2 μg to 7 μg. The maximum limits of linearity were not determined.

The use of peroxide-free diethyl ether is important because the alkaloids, particularly thebaine, are susceptible to oxidation during the evaporation state. The diethyl ether should be checked for the presence of peroxides prior to use.

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