SEPARATION AND DETERMINATION OF OPIATES AND DILUENTS IN ILLICIT NARCOTIC MIXTURES*

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SUMMARY

A semi-automated method for the separation and determination of opiates in illicit narcotic preparations is described. The procedure involves resolution of the mixtures on SE-Sephadex ion-exchanger, continuous ultraviolet monitoring of the column effluent and colorimetric assay of lactose and mannitol. Peak elution volumes (V_e) for compounds of forensic importance are presented. The differences in V_e's for the most commonly encountered opiates and diluents are great enough to permit identification and determination with ease. Examples of analyses of contraband preparations are also discussed.

The increasing problem of drug abuse has emphasized the need for methods that will permit the unequivocal separation, identification and estimation of ingredients found in illicit narcotic preparations. These clandestine materials vary in composition; at one extreme a confiscated packet may contain a single component such as quinine, while another may consist of a mixture of substances including heroin, quinine, mannitol, lactose, procaine, etc.

The literature abounds with thin layer chromatography procedures for the resolution of opiates and related drugs[1-3]. These systems, however, are not convenient for the simultaneous identification of commonly encountered inert diluents such as lactose and mannitol. Moreover, elution and quantitation of thin layer chromatography spots frequently is a difficult task.

By combining ion exchange chromatography, continuous effluent monitoring and a few colorimetric assays, total analysis of narcotic mixtures can be achieved with minimum attention and manipulation by the operator.

EXPERIMENTAL

Column preparation

100 g of SE-Sephadex C-25 (coarse cation exchanger) is permitted to swell in 2 l of 0.2 *M* NaH_2PO_4 buffer, pH 4.6, for 24 h at room temperature with constant stirring. Slurried gel, sufficient to furnish a final column height of 40 cm, is added to a 50 × 0.9 cm chromatography tube in one step via a large reservoir fitted to the top of

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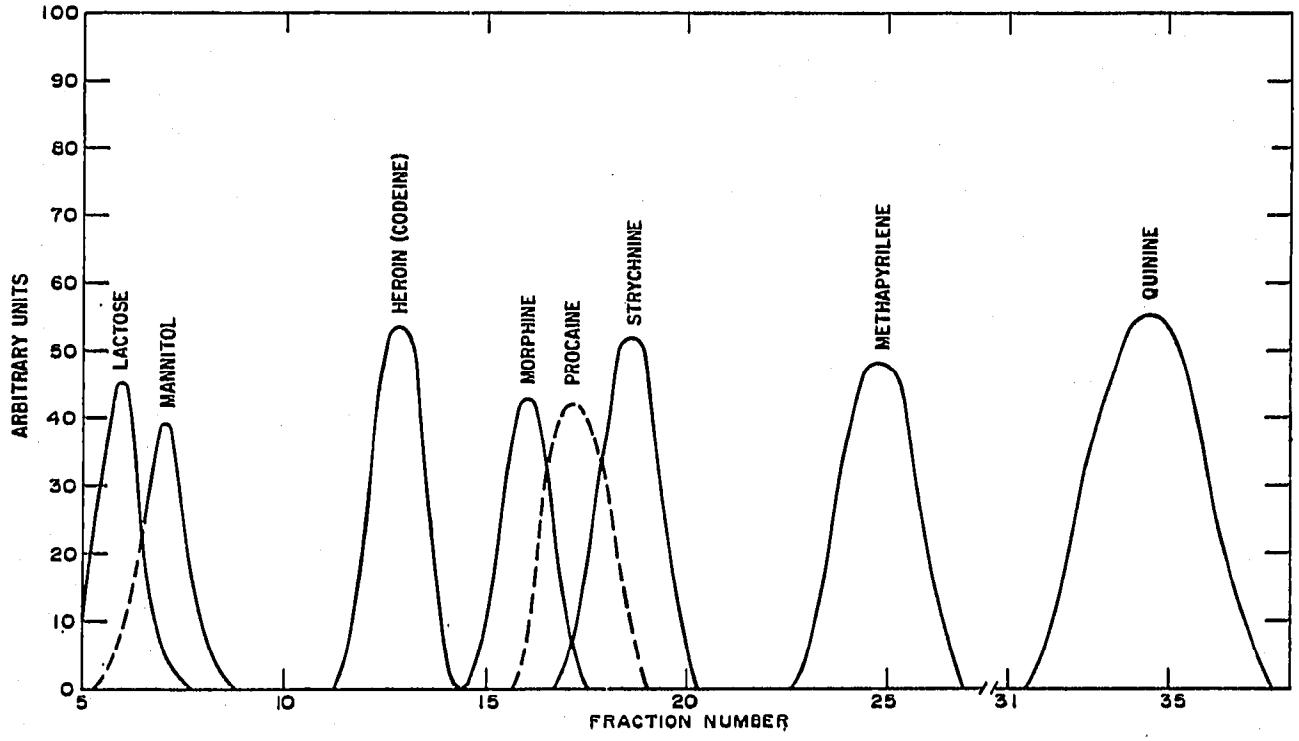

Fig. 1. Separation of compounds of forensic interest using SE-Sephadex cation exchanger.

the tube. The particles are allowed to settle by gravity until a height of about 10 cm is reached. The remaining 30 cm is packed by opening the bottom outlet to permit gentle flow.

Column operation

5 to 10 mg of the substance to be chromatographed is dissolved in 1 ml of the phosphate buffer; 0.1 to 0.3 ml of the solution is placed on the top of the column with a micropipet. The material is washed in with 0.6 ml of buffer in three aliquots of equal volume, taking care not to disturb the gel bed. The tube is capped and eluant buffer is pumped through the column by a Milton-Roy Mini-Pump at a rate of 9.0 ml/h.

Teflon or polyethylene tubing of internal diameter not exceeding 0.066 in. is used to transport the effluent through a Beckman DBG spectrophotometer equipped with a quartz flow cell. The monochromator is set at 240 nm and the spectrophotometric events continuously recorded on a Sargent MR recorder at a chart speed of 0.5 in./h. After leaving the photometer, the effluent is fractionated in an automatic collector at a rate of four fractions per hour. A total of 175–200 ml are so collected to ensure complete elution of material from the column.

Colorimetry

Mannitol is determined by the method of BAILEY[4]. This procedure involves oxidation of the sugar alcohol with periodic acid. After elimination of excess periodate, the formaldehyde is estimated using phenylhydrazine–potassium ferricyanide as the color reagent.

Lactose is assayed by DREYWOOD's[5] anthrone procedure.

RESULTS AND DISCUSSION

Fig. 1 is a composite chart showing the degree of separability among some compounds which might be found in an illicit mixture. The ordinate is plotted in arbitrary units because lactose and mannitol are determined by methods other than ultraviolet absorptiometry. In practice, the ordinate is continuously recorded in transmittance at 240 nm or some other convenient wavelength; peak heights then become valid measurements of the concentrations of those substances exhibiting ultraviolet absorption characteristics.

Peak elution volumes (V_e) for compounds of forensic interest are presented in Table I. The tabulation reveals that overlapping of V_e's for some materials does occur. For example, the difference in V_e's for meperidine and morphine is not great enough

TABLE I

PEAK ELUTION VOLUMES (V_e) FOR COMPOUNDS OF FORENSIC IMPORTANCE

Compound	*Peak elution volume (ml)*
Lactose	27.0
Mannitol	31.5
Heroin	57.6
Codeine	57.6
N-Allylnormorphine	57.6
Ethylmorphine	60.3
6-Monoacetylmorphine	67.5
Narcotine	69.7
Meperidine	71.1
Morphine	72.0
Procaine	76.5
Methadone	77.4
Strychnine	82.8
Succinylcholine	103.5
Methapyrilene	112.6
Papaverine	114.8
Quinine	155.8

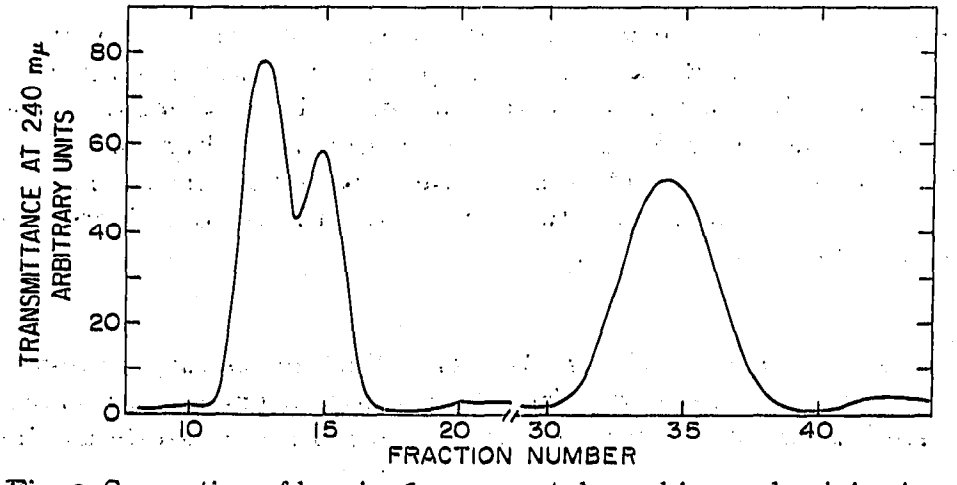

Fig. 2. Separation of heroin, 6-monoacetylmorphine and quinine in a confiscated narcotic preparation.

to provide clean separation of the pair. In instances like this, the elution curve will depart from normal Gaussian shape or will show a distinct shoulder. Non-homogeneous peaks of this kind can be resolved by subjecting small aliquots of the appropriate fraction(s) to thin layer chromatography techniques.

Peak overlap does not seriously diminish the usefulness of the procedure since most seized specimens are composed of easily separated constituents.

For example, FULTON[6] has analyzed 176 contraband mixtures containing heroin. He found 74 % diluted with quinine, mannitol and/or lactose, 20 % with mannitol and/or lactose, and 6 % with quinine and some other contaminant such as procaine or methapyrilene.

Our experience with a limited number (10) of confiscated samples is in substantial agreement with these findings.

We recently were confronted with a sample which was dirty brown in color and gave positive spot tests for the presence of quinine and a morphine derivative.

Fig. 2 shows the tracing observed upon subjecting the mixture to the described method. The peaks at fractions 12.8 and 34.5 (V_e = 57.5 ml and 156 ml, respectively) were confirmed as heroin and quinine. The additional peak at fraction 15 (V_e = 67.5 ml) was identified as 6-monoacetylmorphine. Confirmation was achieved by preparing 6-monoacetylmorphine according to the procedure of WRIGHT[7].

LERNER AND MILLS[8] report that 6-monoacetylmorphine is a common constituent of illicit preparations and results from the use of wet acetic anhydride for the acetylation of morphine and/or hydrolysis of the 3-acetyl group of heroin by moist air.

Subsequent "cutting" with quinine, lactose, etc., does not change the original proportion between heroin and monoacetylmorphine. Consequently, they believe large shipments can be traced from manufacturer to peddler by measuring these ratios.

The method we propose would appear to be eminently suited for this kind of analysis.

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