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Identification and Determination of Heroin in Illicit Seizures by Combined Gas Chromatography-Infrared Spectrophotometry

The identification and determination of heroin in illicit preparations is important especially with regard to providing information for drug intelligence investigations and for dosage regulation in methadone maintenance programs.

A number of reliable methods are available for the identification of heroin in illicit seizures,[3,4,5] however, subsequent quantitation requires extensive manipulation of the sample, especially with seizures such as Brown or Mexican heroin.[4] A rapid procedure has been developed which enables the analyst to routinely identify and quantitate heroin and provides qualitative information concerning a seizure which can be used in drug intelligence investigations. Other diluents in a seizure such as quinine and procaine, can be identified and determined at the same time.

Experimental

Preparation of Samples for Gas Chromatography

Standard reference heroin hydrochloride powder (0.1–10 mg) for the preparation of the calibration curve, or material of an illicit seizure (1–50 mg), for quantitative analysis, were weighed and mixed with a known weight of cholesterol used as an internal standard and then reacted (0.1 ml–1.0 ml) directly with N-O-Bis(Trimethylsilyl)-acetamide in pyridine (BSA/P) (Pierce Chemical Company). The BSA/P is added in excess to eliminate the possibility of hydrolysis due to the presence of water. The reaction mixture was heated at 70 C for 1 min. An aliquot (5–10 μl) of the resulting supernatant was injected into the Barber Coleman model 5320 gas chromatograph equipped with a 6 ft by ¼ in. stainless steel 3 percent OV-17 column on 100/120 WHP (Supelco, Bellefonte, Pa.) a flame ionization detector and a 9:1 effluent splitter. The instrument was operated isothermally at 260 C with a detector temperature of 295 C, an injector temperature of 285 C, and a flow rate of 67 ml/min.

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[3] Clarke, E. G. C., Ed., *Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Postmortem Material*, The Pharmaceutical Press, London, 1969, p. 292.

[4] *Methods of Analysis*, IRS Publication 341, 1967, pp. 55–58.

[5] Shaler, R. C., Jerpe, J. H., Montgomery, R., and Parker, J. M., "Forensic Applications of Combined Gas Chromatography-Mass Spectrometry for the Screening and Identification of Dangerous Drugs and Narcotics," American Academy of Forensic Science Presentation, Phoenix, Ariz., Feb. 1971.

Collection of Samples From the Gas Chromatograph and Subsequent IR Spectrophotometry or Microcrystallography

The heroin was collected from the column effluent by placing an open end capillary tube (100 mm by 1 mm) over the exit port by means of a rubber extension. As the heroin elutes from the column, it condenses on the walls of the coder capillary tube.

Infrared analysis of the collected heroin is accomplished by washing the capillary tube with chloroform, evaporating it around a small amount (1–3 mg) of dry KBr and pressing the mixture into a 1.3-mm pellet. Figure 1 is an example of the IR spectrum which is obtained. The detection limit of the procedure is in the range of 50 to 100 μg.

Microcrystallographic analysis of the collected heroin also can be accomplished by dissolving the drug in a small amount of 0.1*N* HCl and then growing specific heavy metal crystals [*2*]. The detection limit when using microcrystalline procedures is in the range of 1 to 10 μg.

Preparation of the Calibration Curve

The curve was prepared by plotting the ratios of the peak areas of heroin/cholesterol trimethylsilyl derivative versus the ratios of their underivatized weights.

Results

Choice of Internal Standard

The choice of cholesterol-TMS as an internal standard for this system was dependent upon its meeting a number of requirements. (1) Since illicit heroin seizures contain a variety of components which normally elute from the GC column before heroin, an internal standard must necessarily elute after heroin; (2) its elution time must not overlap significantly with that of heroin; (3) the detector response to cholesterol-TMS must be linear with concentration over a wide range; (4) a stock solution should be stable for a considerable period of time in order to permit facile processing of routine samples; and (5) the preparation of its trimethylsilyl derivative must be both quantitative and rapid.

Detector Response to Heroin and Cholesterol

That the detector responds to heroin and TMS-cholesterol in a linear fashion was investigated over a wide concentration range using the procedure of internal standardiza-

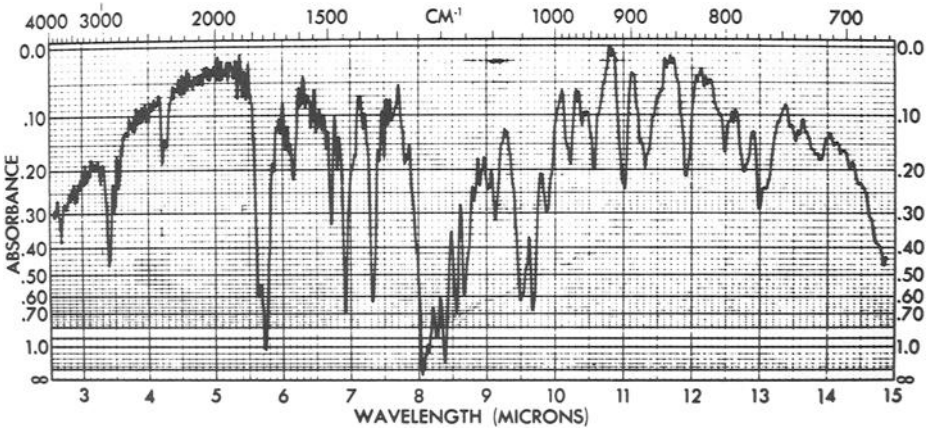

FIG. 1—*Infrared spectrum of heroin free base.*

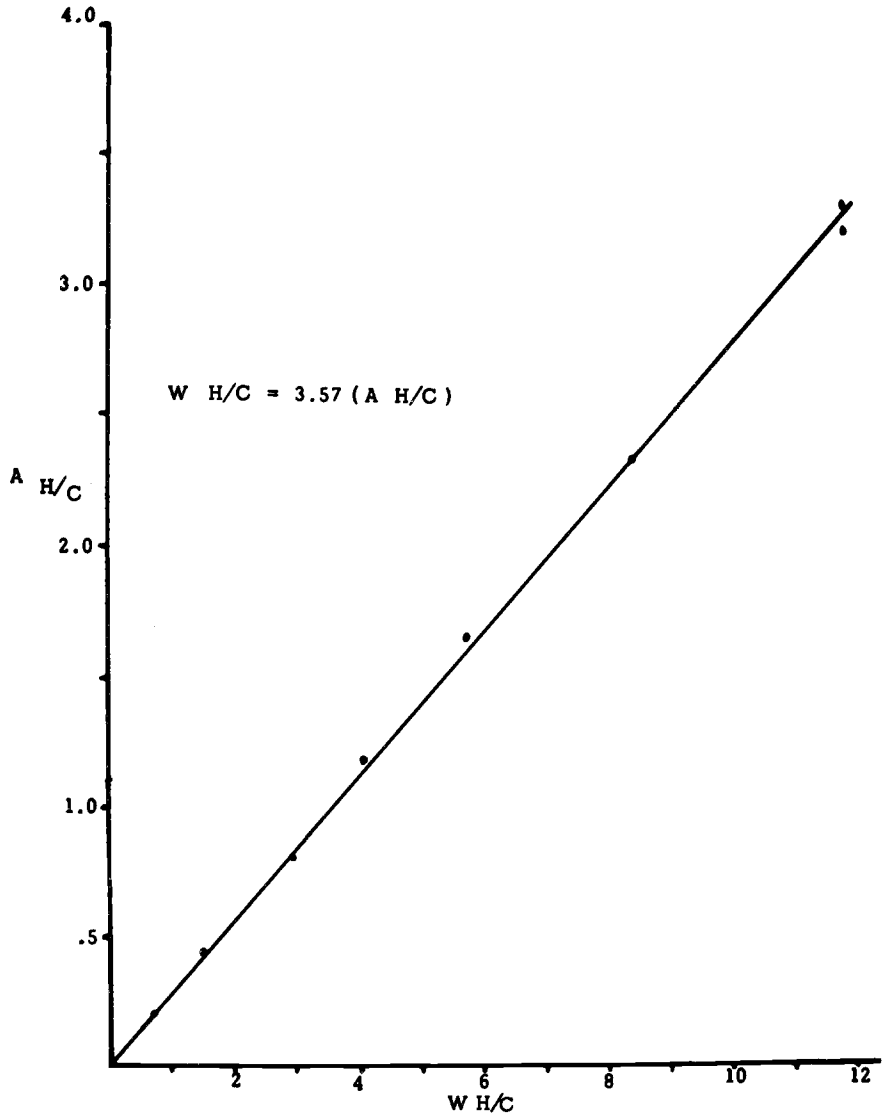

FIG. 2—*Calibration curve for the determination of the weight of heroin in illicit seizures.*

tion.[6] An exact amount of heroin and cholesterol reacted with BSA/P was injected into the gas chromatograph. The calibration curve was prepared as described. The results indicate the linearity of the response over the concentration range investigated (Fig. 2).

Quantitation of Heroin Seizures

Thirty seizures were quantitated for heroin using the GC procedure described and also using recommended spectrophotometric procedures.[4]

[6] McNair, H. M. and Bonelli, E. J., *Basic Gas Chromatography*, Varian Aerograph, Walnut Creek, Calif., 1969, p. 150.

Figures 3*a*, *b*, *c*, and *d* illustrate the results obtained when measured weights of seizure and internal standard are reacted with BSA/P. The illustrations were chosen specifically to illustrate the heterogeneity of the different seizures and the wide application of the method. Figure 3*a* shows the analysis of a typical street heroin seizure wihch contained a large quantity of quinine. The concentration of heroin was 3.9 percent. Figure 3*b* shows the analysis of a seizure of Brown or Mexican heroin. Normally the spectrophotometric procedure of Mexican heroin requires extensive manipulation of the seizure and is extremely time consuming. The percentage of heroin found was 1.5 percent. Figure 3*c* is an example of the analysis of a seizure which contains a small number of diluents and a very low concentration of heroin (1.7 percent). Most of the diluent material was insoluble in BSA reagent. Figure 3*d* shows the analysis of a seizure of suspected Brown or Mexican heroin. The high concentration (16.2 percent) of heroin would indicate that the material is not Mexican heroin. It is likely that the main diluent is brown sugar, or that some Mexican heroin had been "fortified" with some pure heroin.

A wide range of concentrations can be successfully determined by this procedure. Quantitations have been successful on samples containing as little as 1.0 percent to as much as 90 percent heroin.

Comparison of Spectrophotometric and Gas Chromatographic Procedures

Inasmuch as a new method is being established, it is necessary to compare the results with those of an accepted method of determining heroin in illicit seizures. Table 1 compares the results of the gas chromatographic procedure with those of an accepted spectrophotometric procedure. The data included were chosen to illustrate two points: (1) it is generally found that the results of the gas chromatographic method are slightly lower than those of a direct extraction-back extraction spectrophotometric method run on the same sample. This is not surprising inasmuch as heroin seizures are known to contain other material which absorbs ultraviolet light in the neighborhood of the maximum for heroin. Therefore, the instrument cannot distinguish readily between heroin and the other material; and (2) it is also clear that several of the results are widely divergent. In one case, for example (Case 12090) no determination at all could be made using the spectrophotometric technique due to interference with the large amount of quinine present in the sample. Using the GC procedure, the amount of heroin found was 1.5 percent.

TABLE 1—*Comparison of results of gas chromatographic procedure with UV spectrophotometric procedure.*

Case Number	Heroin, % GC	UV
13649	1.9	3.2
13690	5.9	6.0
13708	3.9	4.9
13306	6.3	8.2
13422	1.2	1.0
13493	39.8	41.7
13510	6.5	9.0
13404	6.1	7.5
13591	24.3	21.4
13537	6.5	16.7
12090	1.5	undeterminable
13609	24.3	21.6
2195 (old BSA/P)	2.3	6.3
2195 (fresh BSA/P)	6.8	6.3

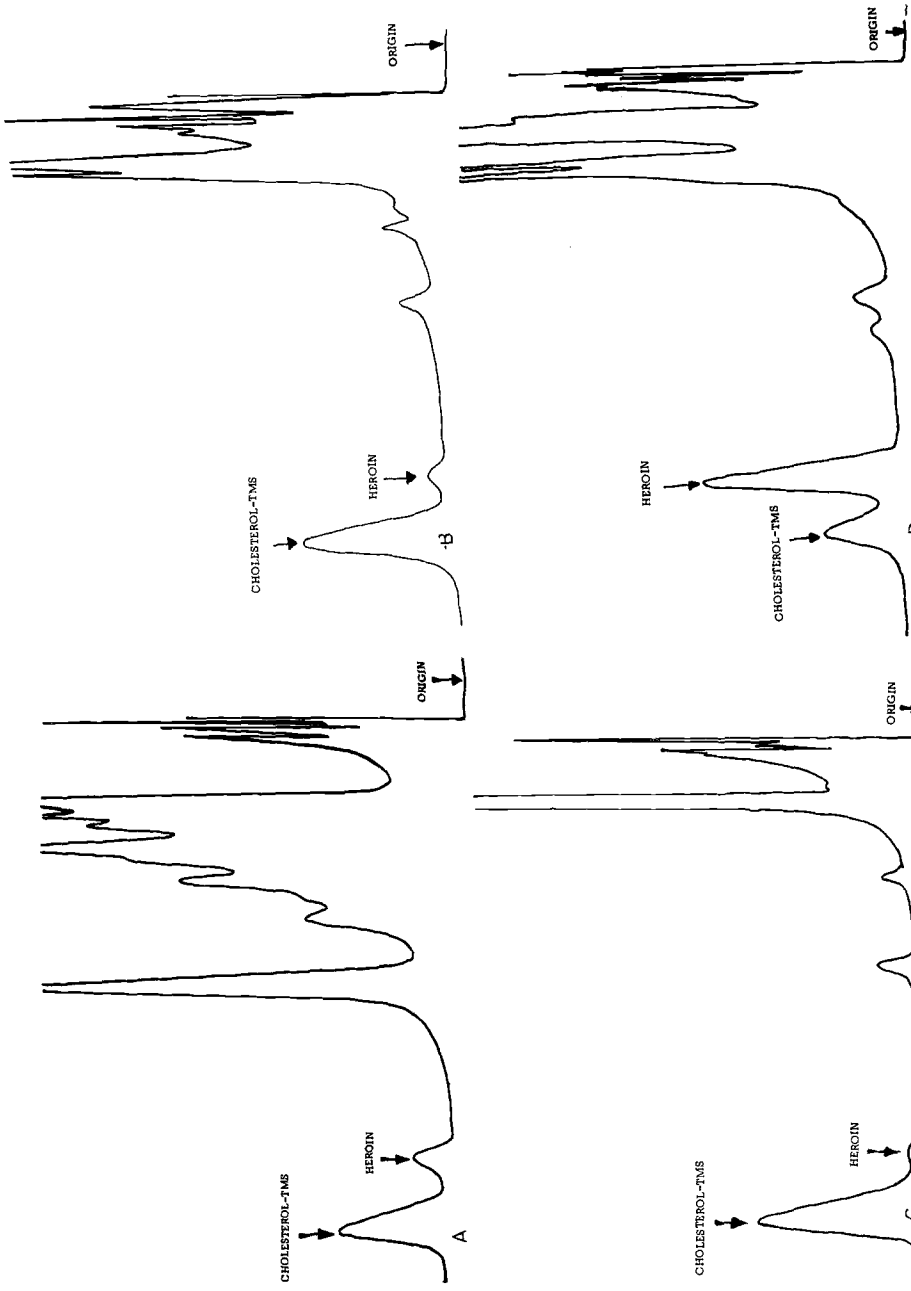

FIG. 3—(a) *Street heroin seizure with large amount of quinine, 3.9% heroin;* (b) *brown or Mexican heroin seizure, 1.5% heroin;* (c) *seizure with mostly insoluble diluent material, 1.7% heroin;* (d) *brown heroin seizure probably not Mexican in origin due to large percentage of heroin, 16.2%.*

Qualitative Analysis of Heroin in Seizures

Qualitative analysis of heroin in seizures is most readily accomplished by growing specific heavy metal crystals.[4] However, sometimes difficulties in growing crystals arise due to the various diluents which are present and the sample must be manipulated so that crystals can be grown or an infrared spectrum or mass spectrum[5] of heroin can be obtained.

In the quantitative procedure which has been described, the heroin is separated from the other constituents of the seizure. The collected peak material can be identified by any one of a number of techniques. In this laboratory the appropriate procedure depends upon the amount of heroin which can be collected. If the GC peak is large enough (that is, ⅓ scale deflection at a sensitivity of 300), the heroin in the capillary tube is recovered by solution in $CHCl_3$ which is then either evaporated onto dry KBr or onto a thallium chloride ATR crystal and identified as the free base by infrared photometric techniques (Fig. 1). If the peak is extremely small, it can still be identified as heroin by rinsing the drug from the capillary tube with a small amount of dilute HCl and growing the appropriate heavy metal crystals. Perhaps the ideal method is to use a combined gas chromatograph-mass spectrometer to identify the mass spectrum fragmentation pattern which is obtained.[5]

Discussion

A procedure for the rapid identification and determination of heroin in illicit seizures has been described. The technique is valuable in that it first reduces the time required to complete quantitative analysis of seizures especially those which are difficult such as Mexican and Brown heroin. Second, it provides for a more precise measurement of the heroin content of seizures in that it inherently eliminates interference from other opiates. This is not true using spectrophotometric procedures since most opiates have an appreciable contribution to the absorption maximum of heroin and may therefore cause significant error in the determination. Third, divergence from ultraviolet data indicates a seizure with an unusual concentration of opiates or diluents which can be used to further classify the material (for example, Case 13537 in Table 1). Fourth, the method provides a "fingerprint" of the seizure which can be used in tracing possible common sources or origin. The qualitative GC tracing used as a fingerprint of the seizure combined with quantitative information about the seizure as well as packaging information can be effectively used to identify similar seizures. Fifth, the quantitative analysis need not be limited to heroin. If the diluents can be identified, their concentrations can also be determined using the same internal standard (cholesterol-TMS). For example, quinine, procaine, methapyriline, and caffeine are common diluents which are used in heroin seizures. A calibration curve can be prepared for each one of these and if they are present in the sample they can also be quantitated. Caution must be exercised since the purity of the peak must be established using identification techniques such as infrared or mass spectrometry. This difficulty has been discussed above.

We also have found that using old (decomposed) BSA/P solutions will cause a wide divergence in the results between the UV and the GC procedures (Case 2195 in Table 1).

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