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The Determination of Excipient Sugar Diluents in Illicit Preparations Containing Heroin by Gas Chromatography

The examination of illicit heroin by the forensic scientist will often entail the identification of adulterants and excipients for intelligence purposes. Fulton [*1*] in an earlier study of adulterant and diluent content of contraband heroin, found quinine, mannitol, and lactose to be the most common diluents. Knowledge of the various diluents used to cut heroin may provide clues that will enable law enforcement agencies to correlate heroin traffic in diverse metropolitan and geographical areas.

Common Chemical Treatments

One of the principal reasons for the continued existence of heroin traffic, as with most other criminal activities, is the extreme amount of profit. A major appeal to narcotics traffickers is heroin's potential for almost unlimited dilution with other substances. It is convenient to distinguish between diluents which are active drug principles, more commonly referred to as adulterants, and inert diluents such as lactose or sucrose. The adulterants are believed [*2*] to be added initially by wholesalers, with further dilution with inert diluents by middlemen and street-dealers in the progression of the trade. Based on the examination of heroin submitted to the Dallas Regional Laboratory, Dallas, Texas, from the central, southwestern, and southeastern United States, quinine and procaine are still the adulterants of choice. Among those who deal in brown, heroin-containing preparations, procaine appears to be preferred to quinine as an adulterant. White, heroin-containing materials also containing quinine have been restricted to the southeastern, and in particular to the New Orleans, area, where they are almost invariably found to also contain mannitol.

While quinine and procaine are the most commonly found adulterants, the forensic scientist must be alert for other drug substances. Methapyrilene has been encountered with increasing frequency in recent years and is now screened for on a routine basis at the Dallas Regional Laboratory. Other adulterants that have been detected in the past are caffeine, salicylamide, tetracaine, acetphenetidin, methadone, amphetamine, antipyrine, aminopyrine, and various barbiturates. The qualitative determination of the active narcotic, associated adulterants, and inert diluents by the forensic scientist will be useful information to enforcement agencies in assessing a peddler's rank in illicit heroin traffic.

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Quantitative analysis of the inert diluents will contribute significantly to the characterization of illicit heroin preparations.

Analysis of the sugars may be especially important in this characterization. A literature survey of current analytical procedures for sugars indicated that few had potential application to narcotic diluent analysis, as most were either too time consuming or lacked the necessary specificity for proper criminalistics analysis. For example, optical crystallography, although an excellent analytical tool for the identification of sugars, is obviously unsuitable where quantitative data are also desired. Broich et al [*3*] simultaneously determined heroin and some of its more common adulterants and diluents by ion exchange chromatography. Continuous effluent monitoring and colorimetric assay were utilized to effect total analysis of narcotic mixtures.

The Application of Gas Chromatography

Gas-liquid chromatographic (GLC) methods have been developed for the rapid analysis of carbohydrates through the conversion of the carbohydrate to a volatile derivative suitable for GLC resolution. The separation and quantitation of carbohydrates and related polyhydroxy compounds by GLC of their trimethylsilyl (TMS) derivatives was described by Sweeley et al [*4*], who silylated sugars to determine the equilibrium composition of anomeric forms. TMS derivatives have since been used extensively in gas chromatographic studies of carbohydrates and sugars in natural products. In a forensic GLC application of TMS derivatives, Grooms [*5*] was able to simultaneously elute heroin, procaine, and lactose on a 5 percent SE-30 column after silyation with N,O-bis-(trimethylsilyl)-acetamide (BSA).

The simultaneous elution of the active narcotic and diluents is useful in comparing illicit heroin samples but can present complications when the alkaloid and diluents have similar retention times. The proximity of the TMS lactose peak to heroin by this procedure necessitates a preferential extraction of the alkaloid when the sugar is present in excessive amounts. Later mass spectrographic studies [*6*] showed that heroin does not react to form TMS derivatives and may consequently also interfere under conditions other than those specifically outlined in Grooms' paper when silyation with TMS derivatives is employed. The use of the sulphur-based solvent pyridine in TMS silyation procedures necessitates its removal prior to injection to avoid unnecessary column and detector maintenance [*7*]. Reid et al [*8*] found that silyl ethers left deposits on the collecting electrodes that required periodic removal to maintain optimum detector performance.

In more recent years trifluoroacetic anhydride (TFA) has been demonstrated to react with 3-phenyl-2-thiohydantoin (PTH) derivatives of amino acids [*9*] and polyhydroxy compounds such as sugars [*10*] to produce derivatives with sufficient volatility for analysis by gas-liquid chromatography. Luke [*11*] has published a method for the determination of sugars in cocoa products which is based on trifluoroacetylation as the derivatizing process. Luke's techniques have been successfully adapted in this study to the total analysis of sugars in illicit heroin. Trifluoroacetic anhydride reacts with sugars to produce derivatives with increased volatility and stability over their TMS counterparts. The TFA derivatives, in particular the disaccharides, have decidedly shorter elution tiimes with no loss in resolution. Heroin and its associated adulterants do not intefere with the gas chromatographic analysis of sugar TFA derivatives; therefore, the separation of these compounds from the sugar diluents prior to derivative formation is not required.

Analytical Techniques

Reagents

1. Acetonitrile
2. Trifluoroacetic anhydride, Eastman organic chemicals
3. Trifluoroacetic acid, sodium salt, Eastman organic chemicals
4. Reaction mixture—Mix trifluoroacetic anhydride with an equal volume of acetonitrile containing 10 percent trifluoroacetic acid, sodium salt. It is recommended that fresh trifluoroacetic anhydride be used for best results as its effectiveness as a derivative agent appears to diminish rapidly after exposure to air. (CAUTION: Prepare reaction mixture using gloves in well ventilated hood.)

Procedure

Place approximately 50 mg of the sample into a beaker and dissolve in a minimum amount of water. Place the beaker in a draft oven set at 100 C and dry to a syrupy consistency. If a draft oven is not available, a steam bath with an air jet will suffice although the drying process will take longer. After cooling the beaker to room temperature, add 3 ml of fresh reaction mixture. Cover the beaker with a watch glass and swirl, with intermittent periods of gentle warming, until the syrupy residue is completely dissolved. This step generally takes about 10 min but can take as long as 20, depending on the composition and amount of sample taken. It should be undertaken in a well ventilated hood while wearing gloves, as trifluoroacetic anhydride and its vapors are hazardous upon contact with skin or inhalation. After derivative formation is complete, transfer the reaction mixture to a volumetric flask with the aid of several acetonitrile rinses. Bring the flask to volume with acetonitrile and mix thoroughly prior to injection into the gas chromatograph. With the detector sensitivity set at half-scale deflection for concentrations in the range of 1 mg/ml, the optimum sample size for analysis, for an injected aliquot of 5 μl, was found to be approximately 50 mg.

The standard stock solution of sugars may be prepared by dissolving the sugars of interest in aqueous solution. A suitable aliquot is then taken for derivative formation. The working reference solution standard was prepared to contain 1 mg/ml of sucrose and mannitol and 2 mg/ml of the reducing sugars dextrose, maltose, and lactose. Although little difficulty was encountered in the derivative formation of actual samples, standard sugar mixtures containing mannitol were slow to dissolve in the reaction mixture. The addition of a small amount of procaine to the aqueous solution before drying greatly facilitated standard sugar derivative formation when difficulties of this nature were encountered. Heroin and associated adulterants appear to serve the same purpose in illicit samples. Standard TFA sugar solutions stored under anhydrous conditions at 0 C are stable for at least one month.

Instrumentation

A Perkin-Elmer 900 gas chromatograph equipped with dual-flame ionization detectors was used. A 6-ft-long coiled glass column, with an outside diameter of $\frac{1}{4}$ in. and an inside diameter of $\frac{1}{8}$ in., was packed with 20 percent SE-30 on 100/120 mesh gas chrom Q and conditioned for 24 h at 250 C. During the operation of this system the nitrogen gas flow was approximately 60 ml/min. The temperature at the injection port was 250 C and at the manifold 270 C.

Graphs plotted for a disaccharide (lactose) and a monosaccharide (sucrose) in units of peak height versus micrograms of sugar injected exhibited linear response and were

reproducible. It was assumed that detector response for other sugars was likewise linear and reproducible.

Experimental

The sugar excipients of 24 samples containing heroin and one sample containing cocaine were analyzed by the gas chromatograph of their TFA derivatives. The results are reported in Table 1. Quantities of heroin and cocaine are calculated as free bases. Adulterants are reported if present, but quantitation was not performed. In a few samples that contained no detectable adulterants, the combined heroin and sugar assay fell far short of the theoretical 100 percent. These samples may have contained substances not detected in their scheme of analysis. One sample had a calculated alkaloid-diluent content exceeding 100 percent, which may indicate the need for an internal standard if exacting precision is required.

TABLE 1—*Sugar excipients of 24 samples containing heroin and one sample containing cocaine.*

	Type of Heroin	Percent Heroin or Cocaine	Area	Adulterants	Sugars or Sugar Alcohols Found
1	White	6.9%	New Orleans, Louisiana	Quinine	6.2% mannitol 46.1% dextrose
2	Brown	8.4%	Denver, Colorado	Procaine	4.4% sucrose 43.3% lactose
3	Brown	8.8%	Houston, Texas	Procaine	26.2% sucrose 11.2% lactose
4	Brown	5.8%	Houston, Texas	Procaine	79.0% lactose
5	Brown	13.5%	Houston, Texas	None found	73.3% sucrose
6	Brown	6.25%	Oklahoma City, Oklahoma	Methapyrilene procaine	55.1% lactose
7	Brown	23%	Houston, Texas	Procaine	56.5% sucrose
8	White	18.55%	New Orleans, Louisiana	Quinine	43.1% mannitol
9	Brown	6.8%	Little Rock, Arkansas	None found	48.8% lactose; dextrose also present
10	Brown	9.5%	Houston, Texas	None found	51.6% sucrose
11	Brown	7.9%	Denver, Colorado	Procaine	30.4% sucrose 19.9% lactose Trace maltose Trace dextrose
12	White	10.5%	New Orleans, Louisiana	Quinine	80.8% lactose Trace mannitol
13	White	29.2%	San Antonio, Texas	None found	37.0% sucrose 16.6% lactose
14	White	11%	New Orleans, Louisiana	Quinine	74.6% lactose Trace mannitol
15	Brown	15.0%	Houston, Texas	None found	65.4% sucrose
16	White	10.4%	New Orleans, Louisiana	None found	28.6% lactose
17	Brown	6.6%	Oklahoma City, Oklahoma	Procaine	40.5% lactose
18	Brown	8.3%	Albuquerque, New Mexico	Procaine	31.2% sucrose
19	White	16.0%	Oklahoma City, Oklahoma	Procaine	26.8% lactose
20	Brown	9.3%	Houston, Texas	Procaine	50.8% lactose
21	White	Cocaine 21.5%	Chattanooga, Tennessee	None found	17.2% dextrose 52.2% lactose
22	White	9.6%	Houston, Texas	None found	40.3% lactose 67.5% sucrose
23	White	12.2%	New Orleans, Louisiana	None found	50.5% mannitol
24	Brown	4.1%	Denver, Colorado	Procaine	3.7% maltose Trace dextrose
25	White	8.4%	New Orleans, Louisiana	Quinine	74.8% mannitol

A synthetic heroin sample composed of 10 percent heroin hydrochloride, 10 percent mannitol, 10 percent quinine sulfate, 10 percent procaine hydrochloride, 20 percent lactose monohydrate, 20 percent sucrose, and 20 percent maltose was prepared and carried through the derivative formation procedure. Gas chromatograms of this solution recorded under both isothermal (Fig. 1) and temperature-programmed conditions (Fig. 2) revealed no extraneous peaks in conjunction with peaks that could be established as sugar TFA derivatives by retention times. Comparison of isothermal chromatograms of this solution with those of a similar solution void of alkaloids indicates that narcotic and adulterant components of illicit heroin do not interfere with or obscure eluting sugar peaks, as found by Grooms [*5*] in his work with TMS derivatives. Thus, while the use of TMS derivatives does produce sugar derivatives of sufficient volatility for analysis by gas chromatography, their volatility is not of the degree of that of the TFA derivatives and will consequently require liquid support phases in the range of 1 to 5 percent. These will, under appropriate conditions, also elute heroin and its adulterants when a nonpolar liquid support phase such as SE-30 is used.

The existence of multiple peaks for both monosaccharide and disaccharide TFA derivatives is an advantage in any identification based upon chromatographic retention times. The reducing sugars maltose, dextrose, and lactose elute as two peaks, since in aqueous solution they exist as mixtures of anomers consisting of alpha and beta forms of hemiacetals. These anomers are in equilibrium, as determined by the solvent used to dissolve the sugar. Derivative formation in a sense freezes these anomers and results in multiple peaks for a single sugar. The combined alpha and beta peaks of dextrose, maltose, and lactose were taken for calculation purposes. All quantitations in Table 1 were based on a ratio of sample to standard peak heights recorded under isothermal temperature

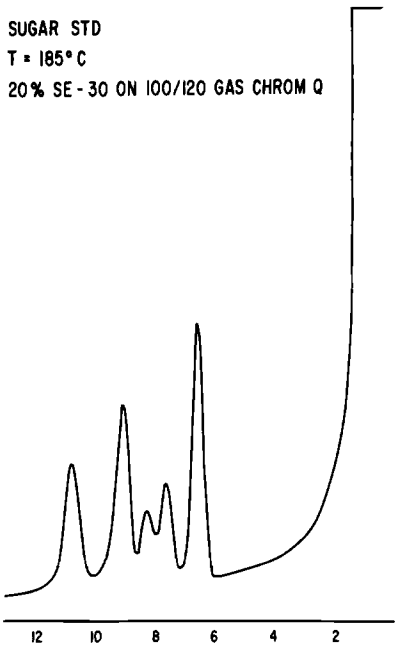

FIG. 1—*Separation of sugars using a 6-ft 20% SE-30 column on gas chrom Q 100/120 mesh at 185 C. Key (letters refer to successive peaks from right to left):* a *sucrose;* b *alpha maltose;* c *beta maltose;* d *alpha lactose; and* e *beta lactose.*

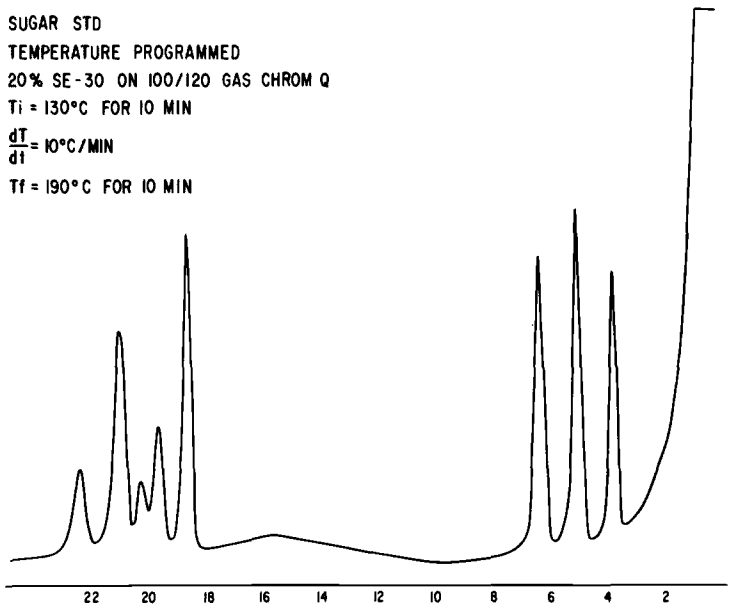

FIG. 2—*Separation of sugars using a 6-ft 20% SE-30 column on gas chrom Q 100/120 mesh under temperature programming. Key:* a *mannitol;* b *alpha dextrose;* c *beta dextrose;* d *sucrose;* e *alpha maltose;* f *beta maltose;* g *alpha lactose; and* h *beta lactose.*

settings. The elution of multiple peaks corresponding to that expected for the anomeric equilibrium of a given TFA sugar derivative yields considerably more evidence than does the retention time of a single peak.

When a quantitative analysis of sugars is not desired, it is possible to accommodate the dissaccharides, monosaccharides, and sugar alcohols all on one chromatogram by carrying out a linear temperature-programmed analysis (Fig. 2). The analyst will then have a gas chromatographic profile of the excipient sugars upon which to base future comparisons. Mannitol and sorbitol have identical retention times but can be easily distinguished by optical crystallography.

Figure 3 is a gas chromatogram of the same control standard on a 2 percent SE-30 column. The solid support employed on all columns made up for this study was 100/120 mesh gas chrom Q. The elution order corresponds to that of the 20 percent column although the alpha and beta peaks of maltose are unresolved. Resolution between the alpha and beta peaks of lactose is also poor. A survey of the literature on TMS sugar chromatographic methods revealed [*12*] that peak resolution and elution times can be primarily dependent on the liquid phase concentration when a nonpolar stationary phase is used. This would also appear to be the case with TFA sugar derivatives. Figure 4 is a chromatogram of the control standard under temperature programming on the 2 percent SE-30 column.

Figures 5 and 6 are chromatograms of the control standard on a 13 percent SE-52 column under isothermal and temperature-programming conditions, respectively. It is evident that SE-52, a slightly more polar phase, also shows promise as a suitable support phase for TFA sugar analysis. The resolution in the initial isothermal temperature plateau under temperature programming, where the sugar alcohols and monosaccharides will

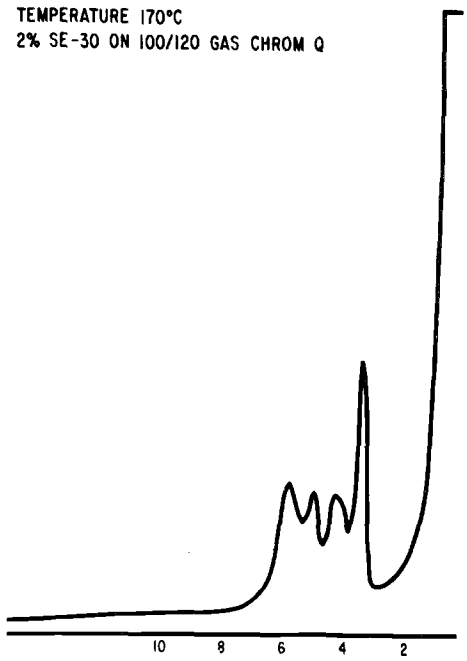

FIG. 3—*Separation of sugars using a 2% SE-30 column on gas chrom Q 100/120 mesh at 170 C. Key:* a *sucrose;* b *alpha and beta maltose;* c *alpha lactose; and* d *beta lactose.*

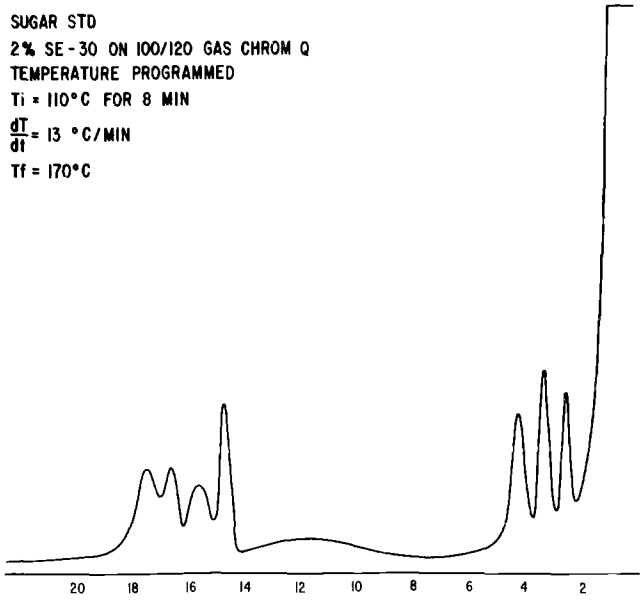

FIG. 4—*Separation of sugars using a 6-ft 2% SE-30 column on gas chrom Q 100/120 mesh under temperature programming. Key:* a *mannitol;* b *alpha dextrose;* c *beta dextrose;* d *sucrose;* e *alpha and beta maltose;* f *alpha lactose; and* g *beta lactose.*

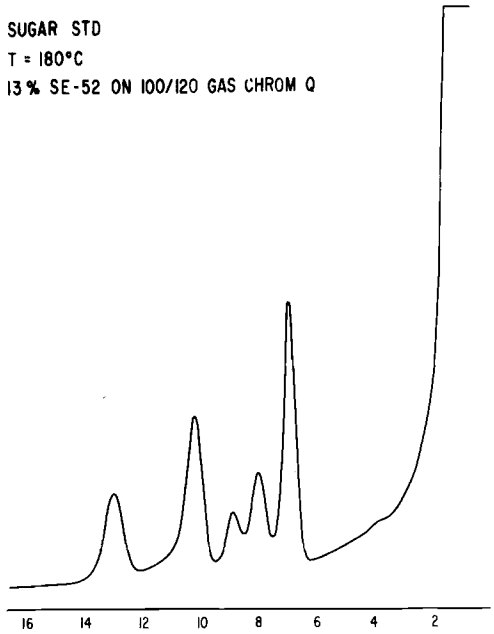

FIG. 5—*Separation of sugars using a 6-ft 13% SE-52 column on gas chrom Q 100/120 mesh at 180 C. Key:* a *sucrose;* b *alpha maltose;* c *beta maltose;* d *alpha lactose; and* e *beta lactose.*

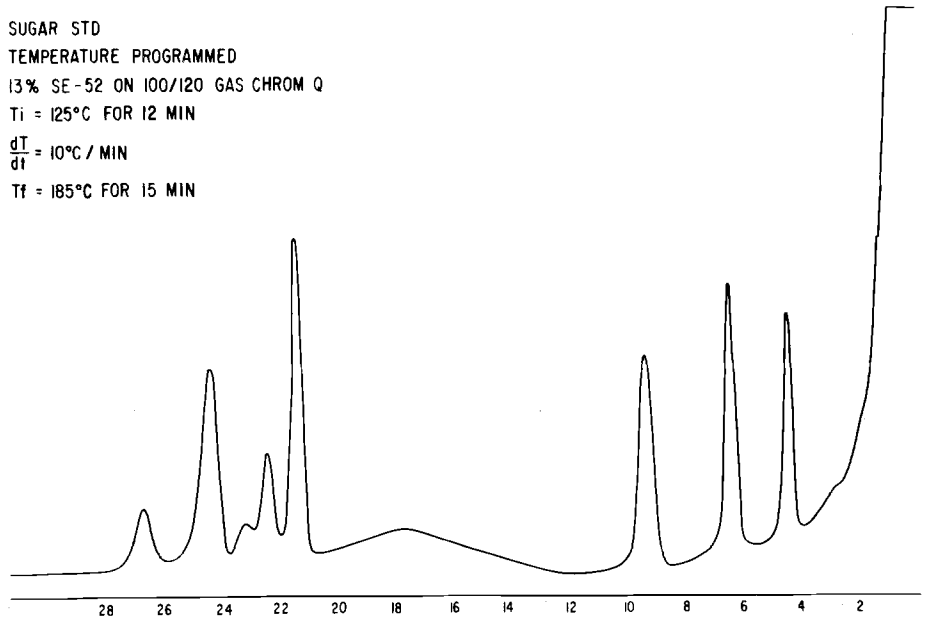

FIG. 6—*Separation of sugars using a 6-ft 13% SE-52 column on gas chrom Q 100/120 mesh under temperature programming. Key:* a *mannitol;* b *alpha dextrose;* c *beta dextrose;* d *sucrose;* e *alpha maltose;* f *beta maltose;* g *alpha lactose; and* h *beta lactose.*

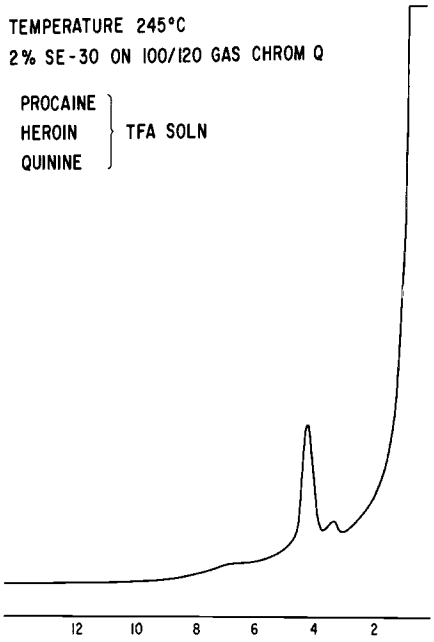

FIG. 7—*Gas chromatogram of TFA alkaloid solution.*

elute if present, is better. This might be the column of choice if less commonly encountered sugars such as the monosaccharides fructose and galactose were suspected to be present.

In an attempt to determine the fate of heroin and adulterants such as procaine and quinine during the TFA derivative process, two solutions containing heroin, procaine, and quinine were prepared at equivalent concentrations of 1 mg/ml. One standard was carried through the derivatizing procedure prior to diluting to final volume. Figure 7 is a chromatogram of the TFA alkaloid solution under the same chromatographic conditions as the unreacted soultion, Fig. 8. One major peak and one minor peak are evident, neither of which have retention times that can be related to procaine, heroin, or quinine. The major peak is suspected to be a derivative of procaine, but no analytical evidence was obtained to support this supposition. The same solutions were subjected to thin layer chromatography in an attempt to establish the nature of the reaction products, with silica gel plus acetonitrile as the developing solvent. Comparison of Rf values for the two solutions established that procaine, heroin, and quinine do react, but the exact identity of these derivative by-products was not determined. In any event, they do not interfere with the analytical determination of TFA sugar derivatives.

Results and Discussion

The analytical results derived from this study would seem to indicate that examination for diluent sugars may not be as routine as perhaps anticipated. Eleven, or 44 percent, of the 25 samples examined contained more than one sugar diluent, which suggests multiple handling. One sample from the Denver, Colorado, area (Fig. 9) was found to contain four sugars, two of which were at such low concentrations that it is doubtful they would have been detected by more conventional techniques of analysis, such as optical crystallography

or simple color tests. As might be expected, the most common pairing of sugars was found to be lactose and sucrose, both of which are readily accessible in bulk at low cost. Other combinations identified in the study were mannitol-dextrose, mannitol-lactose, mannitol-sucrose, dextrose-lactose, and dextrose-maltose.

Figure 10 is a chromatogram of an exhibit from the Denver, Colorado, area that indicated 4.7 percent heroin (expressed as the hydrochloride), 3.7 percent maltose, a trace of dextrose, and a copious quantity of procaine. The similarity in assay values of heroin and

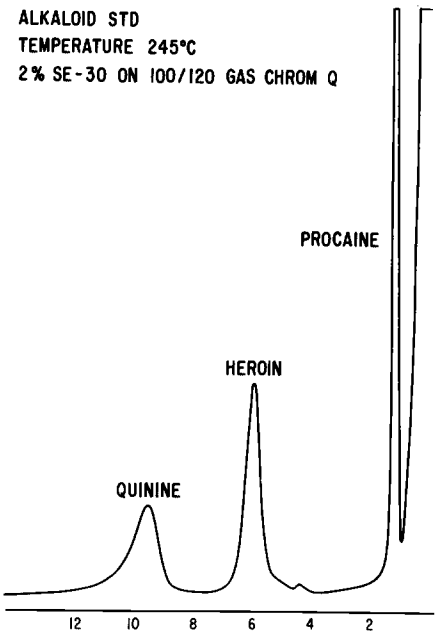

FIG. 8—*Gas chromatogram of unreacted heroin solution.*

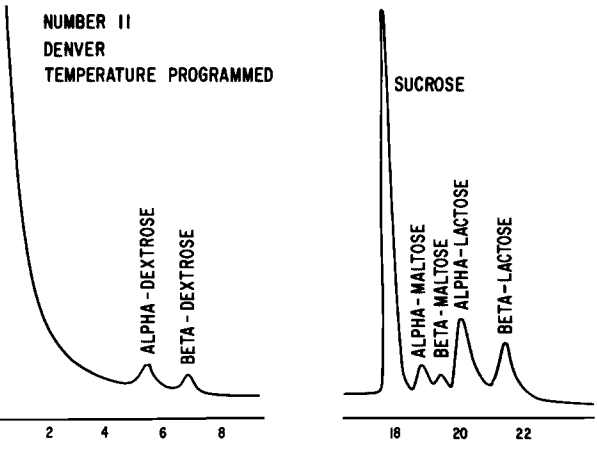

FIG. 9—*Separation of diluent sugars using a 6-ft 20% SE-30 column on gas chrom Q 100/120 mesh under temperature programming.*

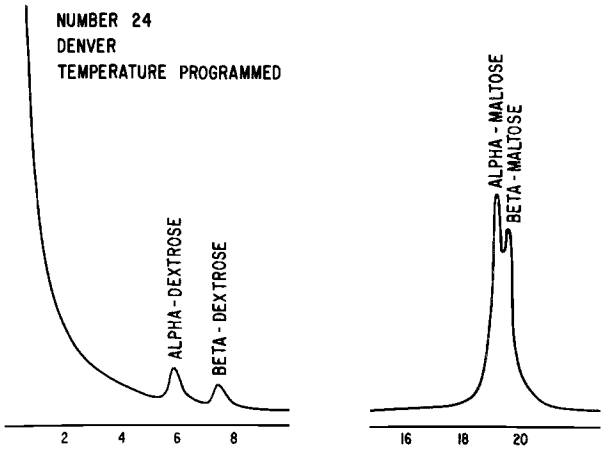

FIG. 10—*Separation of diluent sugars using a 6-ft 20% SE-30 column on gas chrom Q 100/120 mesh under temperature programming.*

maltose suggests that the first cut was made on a 1 to 1 basis with maltose, with a subsequent dilution with procaine to produce a final heroin content of only 5 percent. The exceedingly small amount of dextrose found would indicate that it was not used as a diluent per se but was more probably a contaminant in the dealer's maltose supply. It is interesting to note that two out of three samples selected from the Denver area contained maltose in trace amounts. Figures 11, 12, and 13 are illustrative of the diluent combinations seen in the New Orleans area.

The chronology of hypothetical diluent addition is of course simplified when only one diluent is detected. As an example, two related brown, heroin-containing exhibits were found to contain sucrose as the only diluent (Fig. 14). The percent content of heroin in the original product was calculated by subtracting the sucrose assay from 100 percent. The results was found to be in general agreement with that calculated from the dealer's mixing ratio, as testified to by an undercover agent who was present when the narcotic mixture was prepared.

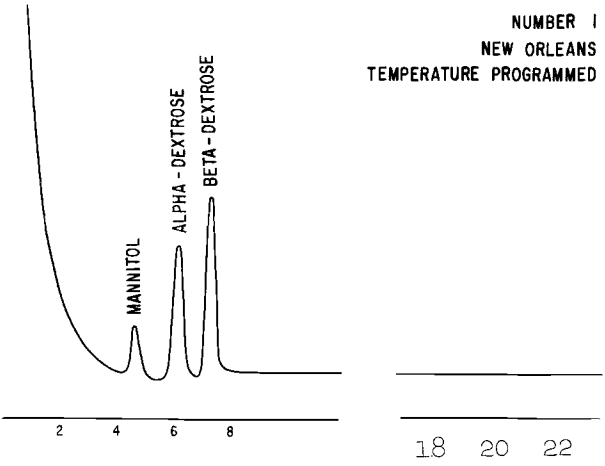

FIG. 11—*Separation of diluent sugars using a 6-ft 20% SE-30 column on 100/120 mesh gas chrom Q under temperature programming.*

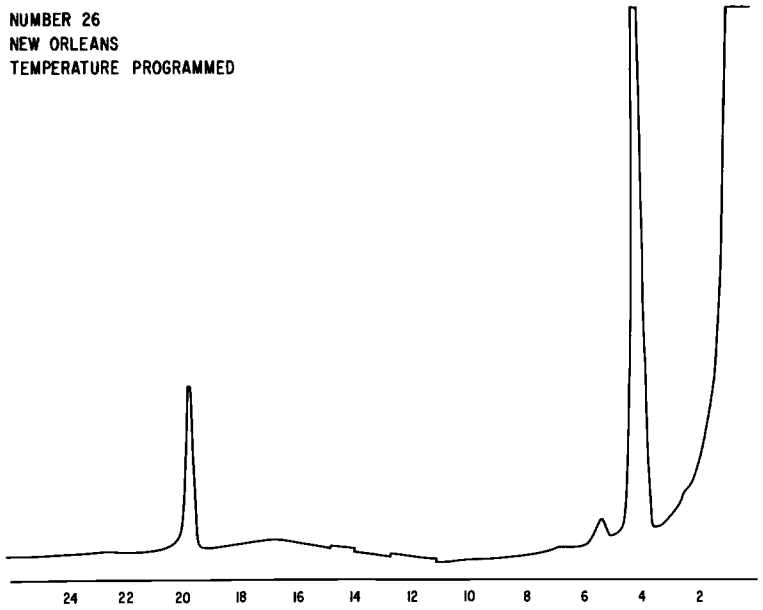

FIG. 12—*Separation of diluent sugars using a 6-ft 20% SE-30 column on 100/120 mesh gas chrom Q under temperature programming. Key:* a *mannitol*; b *sucrose.*

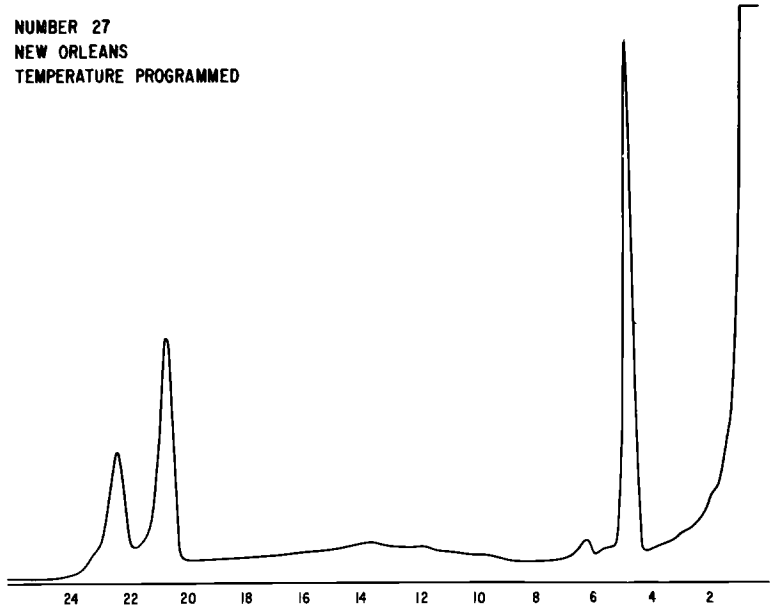

FIG. 13—*Separation of diluent sugars using a 6-ft 20% SE-30 column on 100/120 mesh gas chrom Q under temperature programming. Key:* a *mannitol;* b *alpha lactose; and* c *beta lactose.*

EXHIBIT 1		EXHIBIT 2
Assay:	15.5% Heroin HCl	17.2% Heroin HCl
	73.3% Sucrose	65.4% Sucrose

100% -- Percent Sucrose = Percent Brown Heroin

Exhibit #1 100% - 73.3% = 26.7%

Exhibit #2 100% - 65.4% = 34.6%

Agent's Testimony

16 parts Brown Heroin + 34 parts Sucrose = 50 parts total

Approximate percentage Brown Heroin = 16/50 x 2/2 = 32/100 = 32%

FIG. 14—*Calculations indicating common origin of heroin-containing samples having one diluent.*

Summary

The increasing problem of heroin abuse has emphasized the need for methods that will permit the separation, identification, and estimation of diluents found in illicit narcotic preparations. The total analysis of sugar excipients in illicit heroin can be performed by any laboratory that is equipped for undertaking gas chromatography. Characterization of samples containing heroin by elemental composition [*13*] is perhaps the ultimate in this kind of examination but requires expensive instrumentation that laboratories with limited funding cannot easily afford. A gas chromatographic analysis of sugar diluents is offered as an alternate approach to those laboratories interested in providing this information.

Acknowledgments

I wish to thank Stanley Schreiber of this laboratory for his assistance in the optical crystallographic work performed during the study.

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