Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, Nos 6–8, pp. 813–819, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Comprehensive gas chromatographic analysis of heroin street samples*

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Abstract: A comprehensive analytical approach for heroin street samples is faced with problems associated with differences in polarity, stability and physico-chemical properties of the various substances present. Difficulties with carbohydrates are of particular concern. In this method, which is suitable for screening purposes, 10 mg of sample is dissolved in acetonitrile–trifluoroacetic acid in the presence of methyl orange. Derivatization is then accomplished by *O*-silylation (MSTFA) and *N*-trifluoroacetylation (MBTFA). Compounds were detected by flame ionization after a capillary gas chromatographic separation and produced well shaped peaks for the majority of substances. Sugars gave multiple but reproducible chromatographic peaks. By direct derivatization of another aliquot of the solid sample a single predominant chromatographic peak can be obtained for sugars using MSTFA with MBTFA. Alternatively the more potent reagent MSHFB gives highly reproducible results.

Keywords: *Heroin samples; capillary gas chromatography; trimethylsilylation; trifluoroacetylation; sugars.*

Introduction

A knowledge of the composition of heroin samples is important in order to establish the origin of the drug as well as having medical implications following its use. Many analytical methods have been described to analyze sample content including thin-layer chromatography [1–3], gas chromatography [4–8] and high-performance liquid chromatography [9–13]. Chromatographic methods are required because of the complexity of commonly encountered heroin samples. In addition to the drug, other opiate alkaloids are normally present with various degradation products, and the possibility of many substitutes also being present must be considered. The main added components, however, are substances used to dilute the active principles, especially carbohydrates (monosacharides and disacharrides). The variable physico-chemical properties of these multicomponent samples has made the development of a single comprehensive analytical procedure very difficult to achieve.

*Presented at the "International Symposium on Pharmaceutical and Biomedical Analysis", September 1987, Barcelona, Spain.

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The goal of this work has been to develop a simple screening procedure that permits the simultaneous detection of opiates, adulterants and diluents by means of capillary gas chromatography.

Experimental

Samples

Pure standards of several opiates and cocaine were obtained from the Spanish Ministry of Health (Servicio de Restricción de Estupefacientes y Sicotropos), and other standard drugs were kindly donated by pharmaceutical companies. Authentic street samples of heroin were obtained from patients showing clear clinical signs of heroin overdose, admitted to the Emergency Room of the Hospital del Mar (Barcelona, Spain).

Sample preparation and manipulation

(a) *Direct analysis.* A 10 mg sample was dissolved in 5 ml methanol using an ultrasonic bath (5 min) and 3 μl of the supernatant was injected into the gas chromatograph after centrifugation.

(b) *Selective derivatization.* Sample or standard (10 mg) was dissolved in a mixture (0.5 ml) containing acetonitrile–trifluoroacetic acid (60:40%, v/v) and 200 ppm of methyl orange. The derivatization reaction was carried out as described by Donike [14] with control of the TMS/TFA ratio. *N*-Methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) was added dropwise to the solution until the colour changed from red to yellow. After heating for 5 min at 80°C, 10 μl *N*-methyl-bis-trifluoroacetamide (MBTFA) was added and heating continued for a further 10 min. A volume of 3 μl was then injected onto the gas chromatograph.

(c) *Complete silylation.* For confirmation of sugars a dried sample of 10 mg was directly derivatized with MSTFA (100 μl) for 10 min at 80°C followed by catalysis by MBTFA (20 μl) for 5 min at the same temperature.

Alternatively, *N*-methyl-*N*-trimethylsilyl-heptafluorobutyramide (MSHFB) was added (50 μl) to the dried powder as the only reagent. The sample was then heated for 5 min at 80°C and diluted with ethyl acetate (0.5 ml) before injection.

Chromatography

Separation was carried out, using a 30 m 530 μm SE-30 (1.5 μm film thickness) capillary column (Supelco Inc., Bellefonte, PA, USA) in a Hewlett–Packard model 5890A gas chromatograph equipped with a flame ionization detector. Injector (operated in a split mode, ratio 1:10) and detector temperatures were 250 and 280°C, respectively. Oven temperature was programmed from 150 to 200°C at a rate of 20°C min^{-1} and from 200 to 310°C at 5°C min^{-1}. The final temperature was maintained for an additional 5 min period. Helium was used as a carrier gas at a flow rate of 8 ml min^{-1}. Chromatograms were recorded on a 3392A model Hewlett–Packard integrator.

Results

The direct injection of underivatized standards or street samples allows the detection of a large number of drugs of abuse. The retention times of substances potentially

present in some specimens are listed in Table 1. A representative chromatogram of a mixture of some of these products as pure standards is presented in Fig. 1a. Other compounds bearing polar functional groups need derivatization in order to be suitably analyzed by GC. Our procedure involves trimethylsilylation of hydroxyl groups and trifluoroacetylation of amino groups (see Table 1). Figure 1b shows the chromatogram obtained with the same standard mixture as in Fig. 1a after selective derivatization. Carbohydrates present as diluents in normal street samples are analyzed by gas chromatography after trimethylsilylation. This is accomplished by direct reaction of the solid sample with either MSTFA/MBTFA or MSHFB. Generally one derivative is obtained for those sugars also included in Table 1. Multiple peaks are found, however, when the MSTFA/MBTFA reaction is carried out after sample dissolution in acetonitrile–trifluoroacetic acid. As shown in Fig. 2a, some sugars such as sorbitol could be completely silanized after 60 min at 80°C using MSTFA alone as the silylating agent. When derivatizing the same sugar with MSTFA/MBTFA, the same result was obtained after 15 min. For glucose (Fig. 2b) the addition of MBTFA is very important in order to obtain a single major silanized peak. The same result can be obtained with MSHFB alone, and potential catalysis by *N*-methyl-bis-heptafluorobutyramide (MBHFB) does not improve the yield. The detection of diluents in street samples is illustrated in Fig. 3 where a heroin sample was injected either directly from a methanolic solution (Fig. 3a) or after derivatization with MSHFB (Fig. 3b). The presence of glucose and lactose is

Table 1
Retention times (min) of underivatized and derivatized (selective derivatization with MSTFA/MBTFA) reference compounds. Retention times of sugars correspond to the silanized derivatives, either by using MSTFA/MBTFA or MSHFB

Compound	Underivatized	Derivatized
Heptaminol	3.79	7.04
Amphetamine	3.99	8.86
Ethylamphetamine	5.18	7.67
Phenylpropanolamine	6.12	7.96
Ephedrine	6.75	9.08
Methoxyphenamine	6.87	9.21
Methylephedrine	7.34	9.67
Etaphedrine	8.27	9.67
Caffeine	12.91	ND
Sorbitol	—	14.19
Fructose	—	13.86
Lidocaine	14.24	15.53
α-Glucose	—	15.83
Manitol	—	16.26
β-Glucose	—	17.33
Procaine	16.50	17.96
Methadone	19.06	ND
Cocaine	19.77	ND
Codeine	23.05	24.16
Morphine	23.65	25.52
Ethylmorphine	24.31	25.27
Monoacetylmorphine	25.31	27.56
Heroin	27.01	ND
Strychnine	29.58	ND
Lactose	—	33.30
Sucrose	—	33.85

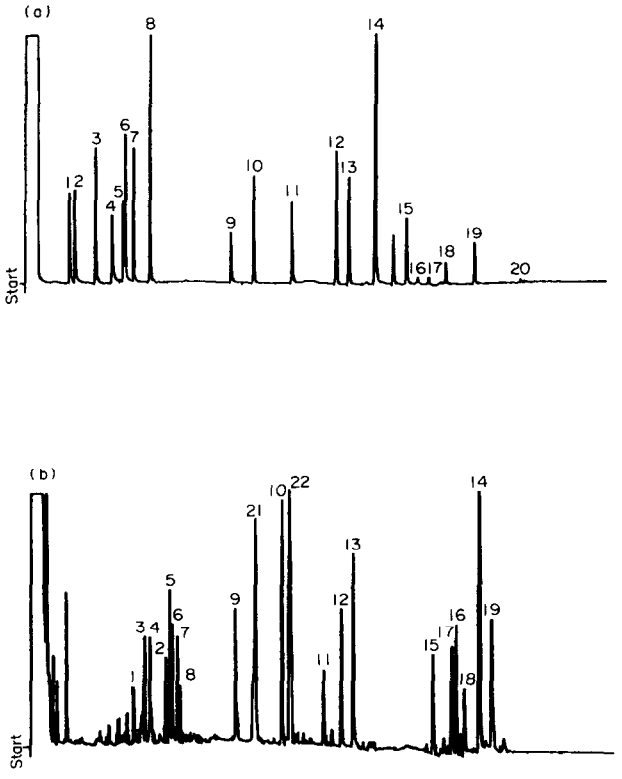

Figure 1
Chromatogram of a mixture of some selected compounds (100 ng each) before (a) and after (b) selective derivatization with MSTFA/MBTFA. 1 = heptaminol; 2 = amphetamine; 3 = ethylamphetamine; 4 = phenylpropanolamine; 5 = ephedrine; 6 = methoxyphenamine; 7 = methylephedrine; 8 = etaphedrine; 9 = caffeine; 10 = lidocaine; 11 = procaine; 12 = methadone; 13 = cocaine; 14 = pentazocine; 15 = codeine; 16 = morphine; 17 = ethylmorphine; 18 = monoacetylmorphine; 19 = heroin; 20 = strychnine. Sorbitol (21) and Manitol (22) were derivatized as dry samples and injected together with the derivatized mixture.

clear after trimethylsilylation. Other polar components such as morphine also become more easily detectable with a well shaped chromatographic peak after the derivatization process.

Discussion

Some information can be obtained by the simple process of dissolving a sample in methanol and subjecting it to gas chromatographic screening (Fig. 3a). This process has considerable limitations, however, as one obtains no information regarding the presence of sugar diluents. Some opiates (i.e. morphine) and polar adulterants (i.e. ephedrine) are not easily detected or quantitated when present in small amounts due to poor chromatographic behaviour.

Conventional derivatization techniques using silylation reagents are useful in handling hydroxyl, phenolic or primary amino groups, but the derivatization of secondary amines is more difficult. The selective derivatization used here [14] produces *O*-trimethylsilyl

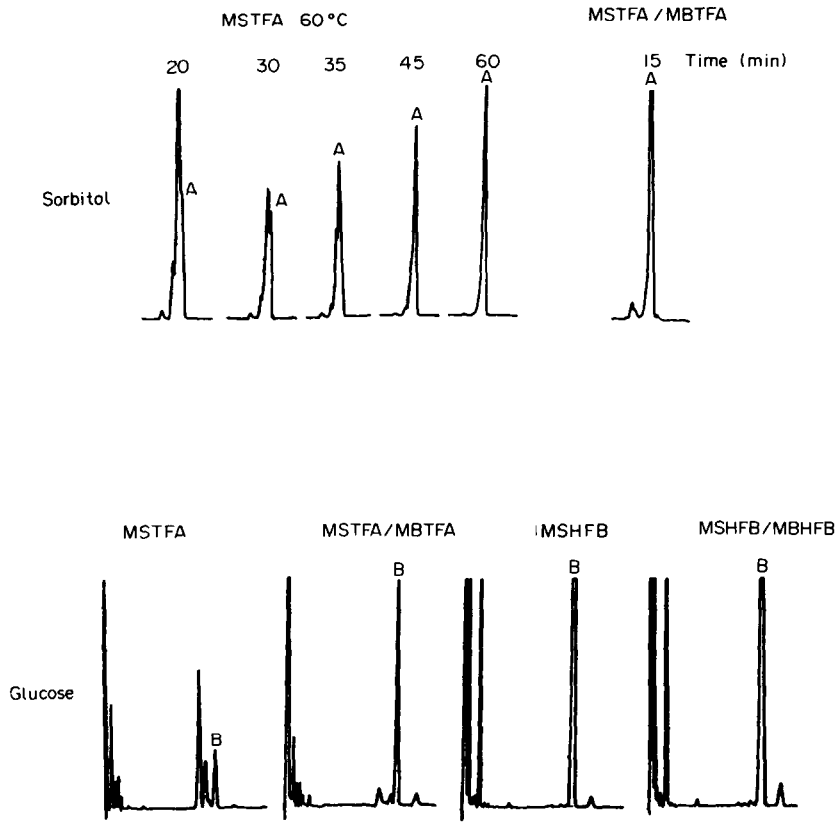

Figure 2
Effect of time and reagents on the trimethysilylation of: (i) Sorbitol; (ii) glucose where A and B are the derivatized materials.

derivatives of primary and secondary hydroxyl groups and *N*-trifluoroacetyl derivatives of primary and secondary amino groups. Extraction of the derivatized compound before injection, which is common in many derivatization procedures [5] is not necessary and makes our procedure easier to carry out.

The use of *N*-methyl-*N*-trimethylsilyl-fluoroalquilamines permits the derivatization of many of the sugars commonly used as diluents in heroin specimens, although it gives rise to multiple, but reproducible, chromatographic peaks. When the reaction is carried out on the sample powder with MSTFA alone, the formation of a single derivatized peak occurs for some compounds (i.e. fructose), whereas others e.g. glucose (Fig. 2b) are not easily silanized. A single peak of each compound was obtained only after the addition of MBTFA which acts as a catalyst. A more direct and rational approach for the detection of carbohydrates is the use of a single silanization reagent such as MSHFB. It has a greater silanization potential than MSTFA [15] and allows a complete derivatization of mono- and di-saccharides to form single chromatographic peaks without the need of a catalyst. Therefore a three step method is proposed for the analysis of street samples. Screening should be carried out both by direct analysis of the methanolic solution (Fig. 3a) and also by injection of the products of selective derivatization (MSTFA/MBTFA)

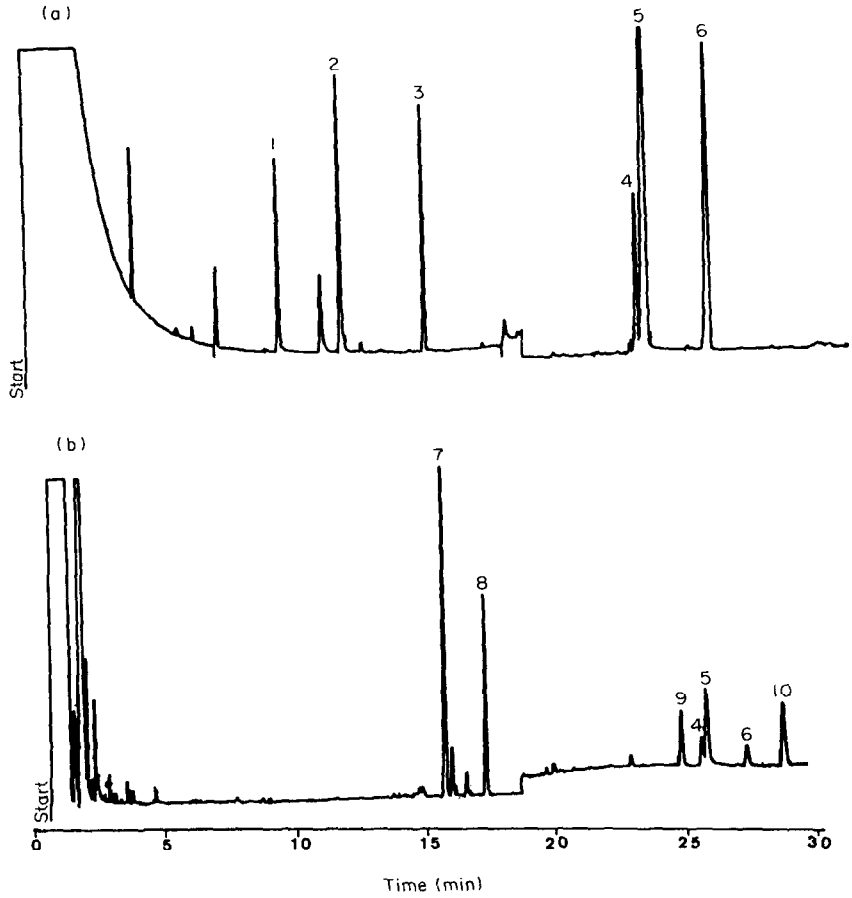

Figure 3
Chromatogram of a street sample of heroin before (a) and after (b) derivatization with MSHFB. [1 = caffeine; 2 = antipyrine; 3 = procaine; 4 = acetylcodeine; 5 = acetylmorphine; 6 = heroin; 7 = α-glucose; 8 = β-glucose; 9 = morphine; 10 = lactose.]

after dissolution of the specimen in acetonitrile-trifluoroacetic acid (Fig. 1b). Confirmation of carbohydrates detected should be performed after direct derivatization of the dried sample, preferably with MSHFB (Fig. 3b).

Acknowledgement — Financial assistance from the Fondo de Investigaciones Sanitarias de la Seguridad Social (FISS 2086/84) is acknowledged.

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[*Received for review 24 September 1987; revised manuscript received 26 November 1987*]