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Note

Gas chromatographic detection of ecgonine and benzoylecgonine in cocaine

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Cocaine hydrochloride is used in medicine as a topical anesthetic. It also has widespread use as a stimulant in the illicit drug market. During recent analytical investigations into illicitly manufactured cocaine, it was discovered that gas chromatographic (GC) methodology to detect the primary cocaine hydrolysis products was lacking. Cocaine can be hydrolyzed in dilute acid to benzoylecgonine, ecgonine, benzoic acid and methanol[1].

The detection of cocaine hydrolysis products is important in the quality control of pharmaceutical cocaine. A number of papers have described the detection of benzoylecgonine and ecgonine in pharmaceutical cocaine and also in botanical and biological samples[2–7]. These procedures utilized primarily paper and thin-layer chromatographic techniques. Fish and Wilson[8] detected benzoylecgonine in urine by methylation with diazomethane prior to GC analysis. However, in this procedure ecgonine was not determined.

GC was selected as an identification tool for ecgonine and benzoylecgonine because it was believed it would surpass present methodology in sensitivity, speed, and specificity. Due to the highly polar and amphoteric nature of the two degradation products, it was determined that their separation from cocaine by liquid–liquid extraction techniques prior to GC analysis was impractical. Subjection of the sample to direct GC analysis was also difficult due to the very poor chromatographic behavior of ecgonine and benzoylecgonine. Therefore, this study describes a procedure whereby both hydrolysis products are silylated prior to GC analysis using N,O-bis(trimethylsilyl)acetamide (BSA). Both ecgonine silyl and benzoylecgonine silyl possess very good chromatographic properties. The derivatized samples are chromatographed on 10% OV-101 on Chromosorb W-HP and 3% OV-25 on Gas-Chrom Q, using temperature programming. Using this procedure ecgonine and benzoylecgonine can be detected in uncut cocaine samples at levels less than 0.1% and 0.3%, respectively.

EXPERIMENTAL

Reagents and chromatographic materials

The BSA silylating reagent used in this study was supplied by Pierce (Rockford, Ill., U.S.A.). The 10% OV-101 on Chromosorb W-HP (100–120 mesh) and 3% OV-25 on Gas-Chrom Q (100–120 mesh) stationary phases were obtained from Applied Science Labs. (State College, Pa., U.S.A.).

Standards

The cocaine hydrochloride used in this study was supplied by S. B. Penick (New York, N.Y., U.S.A.). Hexadecane, eicosane, tetracosane and triacontane internal standards were obtained from Applied Science Labs. Benzoylecgonine and ecgonine were supplied by the Drug Enforcement Administration.

Apparatus

A Packard Model 7400 gas chromatograph was used for all chromatography.

Sample analysis

A 25-mg sample of cocaine hydrochloride is placed in a 1-ml glass-stoppered test tube and 500 μl of BSA is added. The tube is stoppered loosely and heated at 75° for 10 min with occasional agitation. After derivatization is complete, 3–4 μl of the solution are injected into the gas chromatograph using the following parameters:

Column:	Coiled glass, 4 ft. × 4 mm I.D. (OV-101) Coiled glass, 6 ft. × 4 mm I.D. (OV-25)
Stationary phase:	(a) 10% OV-101 on Chromosorb W-HP, 100–120 mesh (b) 3% OV-25 on Chromosorb Q, 100–120 mesh
Carrier gas:	Nitrogen, 60 ml/min
Detector:	Flame ionization
Air:	500 ml/min
Hydrogen:	50 ml/min
Injection temperature:	275°
Detector temperature:	275°
Column temperature:	Temperature programmed initial temperature: 180° (OV-101), 170° (OV-25) initial hold: 10 min (OV-101), 5 min (OV-25) program rate: 3°/min (OV-101 and OV-25) final temperature: 260° final hold: 5 min
Sensitivity:	3×10^{-9} a.f.s. (OV-101 and OV-25)
Chart speed:	0.2 in./min (OV-101 and OV-25)

RESULTS AND DISCUSSION

Fig. 1 illustrates a gas chromatogram on OV-101 of cocaine hydrochloride containing 3% benzoylecgonine and 1% ecgonine, following BSA treatment. Since temperature programming was used, two internal standards were desirable. It is also apparent from Fig. 1 that the detection level for ecgonine is lower than that for benzoylecgonine. However, benzoylecgonine can still be detected at levels considerably less than 0.3%.

In order to investigate the effect the moisture content of cocaine samples would have on the silylation process, water was added to the sample shown in Fig. 1 and to a commercial cocaine hydrochloride standard at a 20% w/w level. The presence

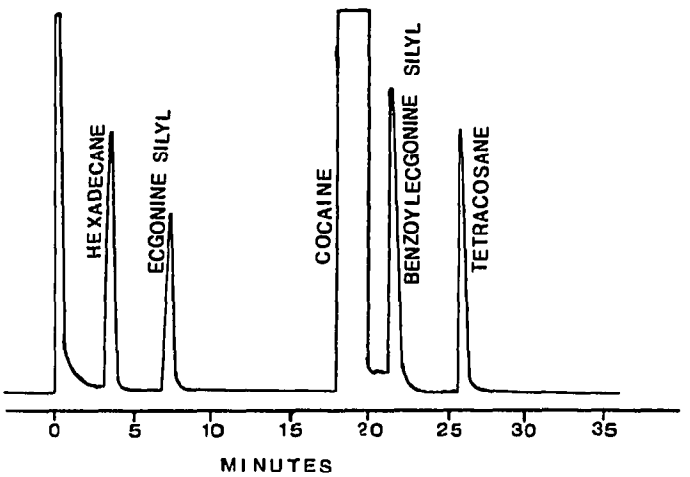

Fig. 1. Gas chromatogram of cocaine hydrochloride containing 1% ecgonine and 3% benzoylecgonine on OV-101 following BSA treatment. See GLC parameters under *Sample analysis*.

of moisture causes no detectable cocaine hydrolysis during the derivatization process. Additionally, the silylation of benzoylecgonine and ecgonine was not affected noticeably. The silylation of both ecgonine and benzoylecgonine is rapid and the BSA solution is stable for at least several hours.

In addition to using BSA derivatizing reagent and OV-101 stationary phase, other silylating compounds and chromatographic stationary phases of varying polarity were investigated. The other silylating reagents included N-trifluoroacetylimidazole and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), the fluorinated analog of BSA. Of these only BSTFA proved of any value. The only advantage in using BSTFA, instead of BSA, was that a slight increase in resolution of the benzoylecgonine and cocaine chromatographic peaks was noted. This was probably due to the relative insolubility of cocaine in BSTFA. However, because of this insolubility, and subsequent loss in benzoylecgonine sensitivity using BSTFA, BSA was selected as the reagent of choice.

In addition to OV-101, 3% OV-1 stationary phase was investigated. However, the resolution between cocaine and benzoylecgonine was less than desirable on this column. More polar phases were also investigated. These included OV-17, OV-25, OV-210, and OV-225. On OV-17, cocaine and benzoylecgonine silyl had the same retention times. When OV-210 and OV-225 were used in conjunction with temperature programming, a rather unstable baseline resulted. OV-25 proved to be the most suitable of the polar stationary phases. When using this phase the elution order of cocaine and benzoylecgonine silyl were reversed when compared to OV-101 (see Table I). The OV-25 was used in this study only as a confirmation for the presence of ecgonine and benzoylecgonine in cocaine. OV-101 was the column of first choice because the resolution, sensitivity and retention times of cocaine and its hydrolysis products were the most favorable. Table I lists retention times of cocaine, benzoylecgonine silyl, ecgonine silyl and internal standards on OV-101 and OV-25. All internal standards were chromatographed using separate chloroform solutions.

The procedure given in this paper is a rapid and sensitive method for the de-

TABLE I

RETENTION TIMES OF COCAINE, ECGONINE SILYL, BENZOYLECGONINE SILYL AND INTERNAL STANDARDS ON OV-101 AND OV-25 STATIONARY PHASES

GLC operating parameters for both columns are given in the text under *Sample analysis*.

Compound	*Retention time (min)*	
	OV-101	*OV-25*
Hexadecane internal standard	3.8	—
Ecgonine silyl	6.7	2.8
Eicosane internal standard	—	5.2
Cocaine	19.7	25.8
Benzoylecgonine silyl	22.2	24.5
Tetracosane internal standard	26.7	—
Triacontane internal standard	—	30.2

tection of small amounts of ecgonine and benzoylecgonine in cocaine. It offers the added advantage of being readily adaptable to quantitative work. The procedure is useful not only in pharmaceutical quality control but in the detection of contaminants in illicitly manufactured cocaine.

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