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Note

Quantitation of cocaine in a variety of matrices by high-performance liquid chromatography

I. JANE, A. SCOTT, R. W. L. SHARPE and P. C. WHITE*
Metropolitan Police Forensic Science Laboratory, 109, Lambeth Road, London SE1 7LP (Great Britain)
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In forensic science laboratories cocaine is rarely encountered as a pure drug. Typically lignocaine and procaine are often added as adulterants, and clandestinely prepared cocaine from "Coca" leaves contains very small quantities of cinnamoyl cocaine. Gas chromatography (GC) can be used for the separation of cocaine from the cinnamoyl cocaine isomers[1], and other synthetic caines provided that there is sufficient material available. Since this method lacks the sensitivity to detect the small quantities of cinnamoyl cocaine present in clandestinely prepared samples of cocaine, the technique is not entirely suitable for confirming the origin of the cocaine. Thin-layer chromatography (TLC) can be used for qualitative analysis of cocaine[2], and UV spectroscopy can be employed to quantitate cocaine provided that there is no cinnamoyl cocaine present. Cinnamoyl cocaine has a very high $E^1_{1\%}$; (360 at 270 nm) in comparison with cocaine ($E^1_{1\%}$; 36 at 270 nm) and therefore traces of the cinnamoyl cocaine can give false quantitative results by UV. The high-performance liquid chromatographic (HPLC) method reported in this paper provides accurate quantitation of cocaine in any matrix and allows discrimination between clandestinely and synthetically prepared samples of cocaine. Both diastereomeric forms of cinnamoyl cocaine were detected and separated by this method of analysis. Methods of preparation of cocaine analogues for use as a reference materials are also reported.

EXPERIMENTAL

High-performance liquid chromatography

Separations were performed on a 15 cm × 4.6 mm I.D. stainless-steel column packed with LiChrosorb RP-2 packing material (5 μm; E. Merck, Darmstadt, G.F.R.). Methanol–0.1 *M* ammonium nitrate (40:60) adjusted to pH 4.3 with 2 *M* hydrochloric acid was pumped through the column at 1.5 ml min^{-1}, and the eluent was monitored at 279 nm with a variable wavelength UV detector (CE 202; Cecil Instruments, Cambridge, Great Britain). Samples were dissolved in the eluent and injected onto the top of the column via a stop-flow injection system[3].

Gas chromatography–mass spectrometry (GC–MS)

A Varian 1400 gas chromatograph (Varian, Palo Alto, CA, U.S.A.) fitted with a 3 ft. × 4 mm I.D. glass column containing 3% OV-17 on Gas-Chrom Q (100–120

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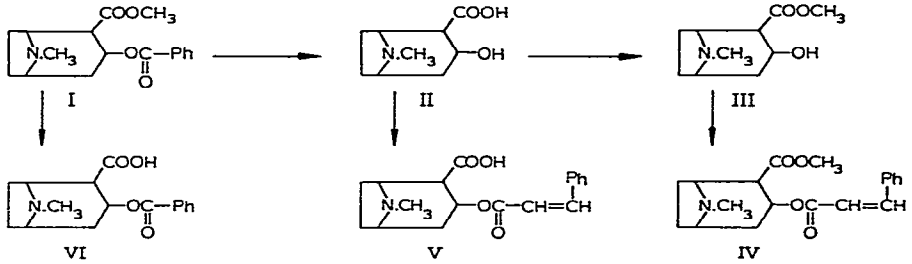
mesh) packing material was linked to a VG Micromass 12-12F quadropole mass spectrometer (VG Analytical, Cheshire, Great Britain). For electron impact studies the separator was maintained at 230°C and the ion source at 200°C and 70 eV. For the chemical ionisation studies isobutane was introduced into the source which was maintained at 170°C and 50 eV with an emission current of 250 μA.

Thin-layer chromatography

TLC was carried out on a 10 × 5 cm plate coated with silica gel 60 F_{254} (E. Merck), and treated with sodium hydroxide. The plates were developed with methanol–acetone (3:1), and the compounds were identified initially by UV adsorption at 254 nm and then by spraying with acidified iodoplatinate solution.

Preparation of compounds related to cocaine

Reference materials were required for this study, and they were prepared as shown below. Ecgonine (II), methyl ecgonine (III), cinnamoyl cocaine (IV), cinnamoyl ecgonine (V) and benzoyl ecgonine (VI) were prepared either directly or indirectly from cocaine (I).



Ecgonine (II)

According to the method of Bell and Archer[4], cocaine (5.5 g) was treated with aqueous hydrochloric acid (130 ml; prepared by adding 10 ml concentrated acid to 150 ml water). The solution was refluxed for 15 h and then extracted several times with ether. The aqueous phase was evaporated to dryness to give a white solid which was recrystallised from ethanol to yield white crystals of ecgonine hydrochloride.

Cinnamoyl cocaine (IV)

Ecgonine hydrochloride (486 mg) in methanol (3 ml) was treated with a solution of diazomethane in ether (5 ml). After 0.5 h at room temperature all the starting material had dissolved. TLC showed a single spot due to methyl ecgonine (III). The solution was evaporated, dissolved in benzene (40 ml) and refluxed for 6 h with cinnamic anhydride (*trans* isomer; 1.83 g) and sodium carbonate (300 mg). The mixture was cooled, filtered and the filtrate was washed with dilute hydrochloric acid (0.5 *M*; 3 × 20 ml). The aqueous phase was basified with ammonia (sp. gr. 0.88) and extracted with ether (3 × 10 ml). The combined ethereal extract was washed with hydrochloric acid (0.5 *M*; 2 × 5 ml). The aqueous phase was saturated with sodium carbonate and a white solid was precipitated. The solid was removed by filtration, dried and recrystallised from light petroleum (b.p. 60–80°C) to give white crystals of cinnamoyl cocaine.

Cinnamoyl ecgonine (V)

Ecgonine hydrochloride (222 mg), cinnamic anhydride (417 mg), benzene (5 ml) and sodium carbonate (100 mg) were refluxed for 2.5 h. After cooling the reaction mixture was filtered and the filtrate was evaporated to give a viscous gum. This was stirred with ether and the ethereal solution was removed and evaporated. The residue obtained was dissolved in ethanol and a small quantity of ether was added, and on standing crystals were deposited. These were filtered off and dried to give cinnamoyl ecgonine.

Benzoyl ecgonine (VI)

Cocaine (250 mg) was refluxed with water (15 ml) for 3 h. The solution was extracted with ether and the aqueous phase was evaporated to dryness. The solid was recrystallised from water to give white crystals of benzoyl ecgonine.

Structures and molecular weights of the prepared compounds were confirmed by GC–MS, and purities were established by GC, HPLC and TLC.

By comparison with the infrared spectra and GC data reported for cinnamoyl cocaine isomers[1], the compound prepared by the above route gave the trans isomer. No cis isomer could be detected by GC or HPLC.

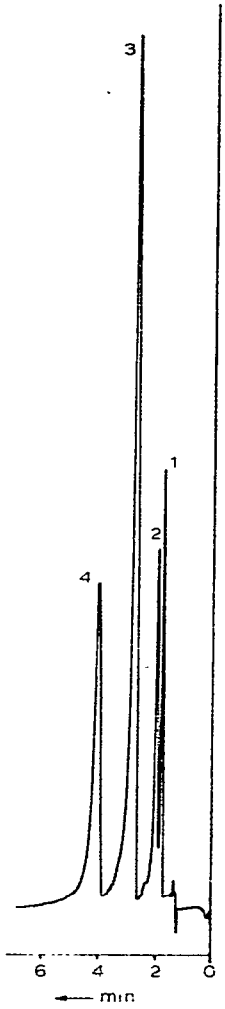
RESULTS AND DISCUSSION

In agreement with several workers[5,6] it was found that weak protolytes *i.e.*, cocaine, were difficult to chromatograph on silica or octadecyl modified silica with aqueous methanol eluents, because their amino functional groups are easily protonated. With both packing materials, methanol concentration, ionic strength and pH affected peak shape and retention time and no suitable combination could be obtained to effect a satisfactory result for the compounds that were to be separated. Following further investigations good chromatographic separations and peak profiles were eventually obtained on a reversed-phase LiChrosorb RP-2 packing material. The separation mechanism for this system is complex, but it is believed that both partition with the bonded portion of the packing material and adsorption on the unreacted surface silanol groups are responsible for the separations. Using the conditions described in the experimental section the separation obtained for a standard containing procaine, lignocaine, cocaine and cinnamoyl cocaine is shown in Fig. 1.

With exception of cinnamoyl cocaine, standards were stable in eluent for several weeks. Cinnamoyl cocaine was found to decompose in this solvent after a few days, but no decomposition was detected when this material was stored in chloroform.

Retention data for the cocaine analogues, synthetic caines and some other drugs that have been detected in cocaine samples are shown in Table I.

Since cinnamoyl cocaine has a relatively high $E^1_{1\%}$ in comparison with cocaine the very low levels of the former compound were easily detected with the UV detector. Discrimination between clandestinely prepared and synthetically produced cocaine was possible because only the clandestinely prepared material contains cinnamoyl cocaine. Of the 336 cocaine samples that were analysed during the last year in this laboratory, 126 were found to contain cinnamoyl cocaine. Lignocaine, procaine and benzocaine were found in 60 of these samples, and mannitol, glucose and lactose were



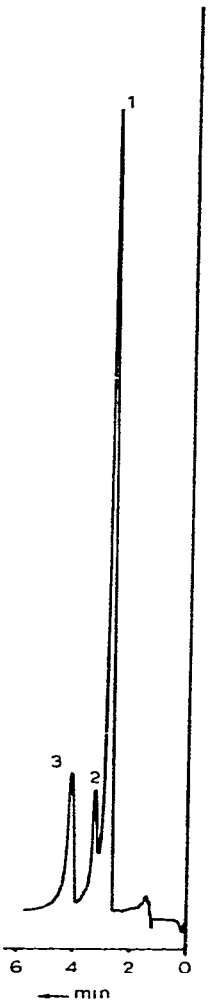


Fig. 1. Chromatogram of a standard mixture. Peaks: 1 = procaine; 2 = lignocaine; 3 = cocaine; 4 = *trans*-cinnamoyl cocaine. Conditions of analysis are as stated in the text.

Fig. 2. Chromatogram showing the components that are commonly detected in clandestinely prepared samples of cocaine. Peaks: 1 = cocaine; 3 = *trans*-cinnamoyl cocaine. Peak 2 postulated as *cis*-cinnamoyl cocaine following preparative HPLC and GC–MS studies. Conditions of analysis are as stated in the text.

detected as diluents in 70 samples. Several samples were found to contain as many as four drugs in combination with cocaine.

In a large number of the clandestinely prepared cocaine samples, another component was detected with an relative retention time of 1.18, and a typical example is shown in Fig. 2.

To help in the characterisation of this material preparative HPLC was employed. By increasing the ionic strength of the aqueous phase to 0.2 *M* ammonium nitrate the component was nearly resolved from the cocaine and fractions were col-

TABLE I

HPLC RETENTION DATA

Relative retention times (RRT values) were calculated with respect to cocaine (retention time 2.7 min)

Compound	*RRT*	*Compound*	*RRT*
Cocaine analogues and synthetic caines		*Alkaloids*	
Procaine	0.65	Morphine	0.45
Chloroprocaine	0.67	Codeine	0.52
Lignocaine	0.70	Monoacetylmorphine	0.61
Pyrrocaine	0.74	Diamorphine	0.91
Benzoyl Ecgonine	0.77	Acetylcodeine	0.92
Dimethocaine	0.77		
Octacaine	0.77	*Basic drugs*	
Propoxycaine	0.80	Ephedrine	0.58
Prilocaine	0.83	Caffeine	0.60
Mepivacaine	0.83	Amphetamine	0.69
Orthocaine	0.89	Methylamphetamine	0.69
Cocaine	1.00	Cyclizine	2.58
Benzocaine	1.13	Dipipanone	2.58
Butanilicaine	1.13		
Piperocaine	1.17		
cis-Cinnamoyl cocaine	1.18		
Leucinocaine	1.26		
Proxymetacaine	1.32		
Amylocaine	1.41		
Butacaine	1.47		
trans-Cinnamoyl cocaine	1.49		
Amydricaine	1.69		
Phenacaine	1.72		
Cinchocaine	3.00		
Cyclomethycaine	3.00		

lected, pooled, extracted and analysed by GC–MS. The spectrum obtained was identical to that of our *trans*-cinnamoyl cocaine, but the retention times were shorter for this unknown by both GC and HPLC. It was postulated that this compound was the *cis*-isomer of cinnamoyl cocaine.

With the analytical conditions described earlier the lower limit of detection was 100 ng for cocaine, and the UV detector response was found to be linear over the range 0.01 to 0.2 a.u.f.s. A relative standard deviation of less than 3% was obtained from peak height measurements for a cocaine standard, and quantitation of cocaine in a sample was therefore obtained by calculating the sample to standard peak height ratio without the use of an internal standard.

Reproducibility of this method of quantitation was established by analysing ten accurately weighed samples of a mixture which contained cocaine. Duplicate injections of these samples were followed by an injection of a cocaine standard, and the purity of each sample was determined by comparing peak heights with that of the standards. A relative standard deviation of 5% was recorded and therefore this method of quantitation was shown to be both reproducible and rapid.

CONCLUSIONS

Using HPLC with UV detection and a LiChrosorb RP-2 reverse phase packing material cocaine could be detected in a variety of matrices. The ability of the system to separate cocaine from cinnamoyl cocaine enables accurate quantitation of cocaine. A method of comparing the peak heights of cocaine in samples against a cocaine standard of known concentration gives rapid and reproducible results.

The detection of cinnamoyl cocaine in samples can be used as a method of discrimination, since only clandestinely prepared cocaine will contain this material. Some samples of clandestinely prepared cocaine were found to contain both isomeric forms of cinnamoyl cocaine which could be easily separated by this method of analysis.

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