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The Rapid Determination of Cocaine and Other Local Anesthetics Using Field Tests and Chromatography

Cocaine, which is obtained from the leaves of plants of the genus *Erythroxylon,* either directly or by chemical transformation, has been used throughout recorded history because of its local anesthetic activity [*1*]. As a result of its undesirable side effects its use is now controlled almost worldwide. The search for alternative compounds having topical or local anesthetic properties without the inherent disadvantage in using cocaine resulted in the synthesis of a number of satisfactory alternatives to cocaine.

In the United Kingdom officers of Her Majesty's Customs and Excise have made many seizures of unauthorized imports of cocaine and materials purporting to be cocaine. A majority of the larger seizures originate from South America (primarily Colombia, Bolivia, and Peru). Many of these are adulterated with uncontrolled local anesthetics (that is, not "Controlled Drugs" subsumed by the Misuse of Drugs Act 1971) and it is not uncommon to analyze a sample that consists wholly of one or more such anesthetics. In this laboratory the most frequently encountered anesthetics used as cocaine adulterants are lignocaine (lidocaine), benzocaine, procaine, and tetracaine. Other workers [*2–4*] also found these to be commonly used for this purpose in the United States. A survey of seizures examined in this laboratory from 1974 through 1978 shows that certain other local anesthetics are also used, although less often. A list of the 14 local anesthetics identified in exhibits submitted to the laboratory during this period is given in Table 1.

Bupivacaine, which is also included in Table 1, has the same gas chromatographic retention time as cocaine. They are, however, readily separated by thin-layer chromatography, and in any event it has not been encountered here in 500 "cocaine" seizures over the same period.

In some seizures the carrier may be a foreign national in transit and in other cases the need for further customs investigation may hinge upon the result of an analysis. Under such circumstances it is necessary to provide a rapid analytical service. The combination of techniques now described has been developed for routine use and has been evaluated on many actual samples.

The procedure is based on a field test, thin-layer chromatography (TLC), and gas-liquid chromatography (GLC). By using these methods, the total analysis time for the quantitation of cocaine and the identification of any admixed local anesthetic, including the preparation of a written witness statement for presentation in court, is approximately 1 h. Because of the wide range of purity of illicit cocaine resulting from adulteration,

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TABLE 1—*Compounds used in this study.* [a]

Compound	Alternative Names
Amylocaine (BPC)	...
Benzocaine [b] (BP)	ethyl aminobenzoate
Prilocaine (BAN) (INN) (USAN)	Propitocaine [c]
Lignocaine [b] (BAN)	lidocaine (INN); Xylocaine [c]
Piperocaine (INN) (BAN)	Metycaine [c]
Tropacocaine	tropacaine
Procaine [b] (INN)	Novocaine, [c] Planocaine, [c] Westocaine [c]
Mepivacaine (INN) (BAN)	Carbocaine, [c] Scandicaine [c]
Tetracaine [b] (INN)	amethocaine (BP), Pontocaine [c]
Cocaine	...
Bupivacaine (INN) (BAN) (USAN)	Marcaine [c]
Proxymetacaine (BAN) (INN)	proparacaine (USP), Alcaine, [c] Opthaine [c]
Butacaine (INN)	...
Cinchocaine (INN)	dibucaine (USP)
Cyclomethycaine (INN) (BAN)	Surfacaine [c]

[a] Abbreviations:
BAN = British Approved Name.
BP = British Pharmacopoeia title antedating BAN list.
BPC = title was official in British Pharmaceutical Codex 1949 monograph.
INN = International Nonpropietary Name.
USAN = United States Adopted Name.
USP = United States Pharmacopoeia title.
[b] Commonly encountered adulterant of cocaine.
[c] Trade name.

poor preparative procedure, or incomplete solvent removal, the analysis must necessarily be quantitative in respect to the cocaine present. In addition, identification of the adulterants present is often helpful to investigating officers.

Cocaine Identification and Analysis

Many different field tests for the preliminary identification of cocaine, whether or not mixed with other local anesthetics, have been described and evaluated [*5-7*]. The reagents used in the present work can be prepared in the laboratory and are available as part of a commercially available drug test kit (BDH Chemicals Ltd., Poole, Dorset, United Kingdom).

Thin-layer chromatography is widely used in the identification of drugs, and its use for the detection of cocaine has recently been reviewed [*4*]. Gas chromatography (GC) has been the method of choice for the quantitative analysis of cocaine, and the separation of cocaine from various other local anesthetics has been described [*8-10*]. Some of these do not give adequate resolution or do not cover the range of compounds of interest in the present study. One of the difficulties in devising a rapid method for the separation of these compounds is that many are structurally unrelated, making selection of appropriate chromatographic conditions rather difficult. Most workers use silicone gum stationary phases, which give rise to well-defined elution profiles but result in a wide range of retention times. The present work describes alternative GC procedures, one a simple isothermal and isobaric system which, although separating all but 2 of the 15 compounds, does not overcome the drawback of excessive analytical time. The second procedure employs the same chromatographic column but involves programming the flow of the carrier gas. While maintaining an adequate separation between the same compounds it achieves a substantial reduction in the time required for analysis. The conventional way

to achieve this is by temperature programming, although the effect on the retention times of the individual constituents of a mixture is somewhat different [*11*].

For consecutive analyses temperature programming has the disadvantage that an equilibration time of at least 15 min between analyses is required. Previous work [*12*] has demonstrated that with flow programming equilibrium time is reduced to about 3 min. Other advantages accruing from flow programming are that stationary phase bleed and the occurrence of ghost peaks, resulting from desorption, are less likely to occur than with temperature programming. However, care must be taken to calibrate the detector because changes in the carrier gas flow rate can affect detector response although reproducibility of results is not impaired [*13*].

Gas chromatography alone is not adequate for the identification of cocaine and a combination of techniques such as those above should always be used. Alternative methods to those described include infrared spectrometry, high performance liquid chromatography [*14*], and mass spectrometry. Infrared data on cocaine and other local anesthetics are available [*15*], and a recent publication [*2*] describes a combination of liquid chromatography and infrared detection. This approach is rather time-consuming if identification of all the compounds is required. Where only trace quantities of cocaine are available, such as in clothing or on scales, mass spectrometric confirmation is essential, but for gross amounts of cocaine resort to such sophisticated equipment is normally unnecessary.

Materials and Equipment

Two field test reagents were used. The first, LSD reagent [*16,17*], consisted of 50 g of 4-dimethylaminobenzaldehyde, 500 ml of methanol analytical reagent, and 500 ml of orthophosphoric acid (minimum 88% purity). The cocaine reagent comprised 8 g of cobaltous thiocyanate, 10 g of phosphorous acid, 400 ml of methanol analytical reagent, and 600 ml of water.

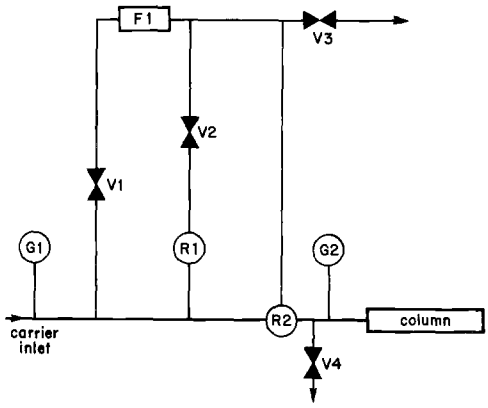
Thin-layer chromatography was carried out on 10- by 20-cm silica gel $60F_{254}$ precoated plates, 0.25 mm thick (Merck, No. 5729), with two solvent systems. System 1 [*18*] was composed of ethyl acetate, methanol, concentrated ammonia (specific gravity, 0.880) in the proportions 85:10:5. System 2 was composed of methanol, 2*N* ammonium hydroxide, and 1*N* ammonium nitrate (27:2:1).

Detection of compounds separated by TLC was by two methods. Method 1 involved inspection of the dried plate under ultraviolet irradiation (nominally 254 and 366 nm), whereas Method 2 involved spraying with iodoplatinate reagent prepared by mixing 2 g potassium iodide, 0.2 g chloroplatinic acid, and 100 ml water. This mixture was acidified before use with two drops of concentrated (12*N*) hydrochloric acid.

Gas-Liquid Chromatography

A Pye 104 gas chromatograph fitted with a flame ionization detector was used. The column was 1.5-m by 4-mm internal diameter glass containing 3% OV-17 stationary phase on Gas Chrom Q (100-120 mesh). The oven temperature was 220°C and detector temperature 300°C.

There are few commercially available flow-programming units, but such a unit can be built in the laboratory with readily available and inexpensive components (Fig. 1). Details have been published [*12*] of a unit combining flow programming with solvent venting. The latter is not required in this instance, which thus enables a simplification of both the construction and operation of the unit. In Fig. 1, Items V1 to V4 are simple on/off values (Drallim) and G1 and G2 are pressure gauges (Budenberg 0 to 414 kPa [0-60 psi]). Items R1 and R2 are diaphragm pressure regulators (Watts, Type 15-2), the latter



Symbol	Description	Function
F1	flow controller	rate of programming
G1	pressure gage	supply pressure
G2	pressure gage	column pressure
R1	pressure regulator	initial pressure limit
R2	pressure regulator	programming regulator
V1	on/off valve	program start
V2	on/off valve	initial pressure reset
V3	on/off valve	pressure release at completion of run
V4	on/off valve	pressure release at completion of run

FIG. 1—*Diagram of flow-programming system.*

modified as previously described [*12*], and F1 is a flow controller (Brooks needle valve, Type 8501) fitted with a digital revolution counter (Brooks, Type 8513). The rate of flow programming is controlled by F1 and monitored, as the resulting pressure change, on G2. Increasing the flow rate of carrier gas into the detector can result in a change in standing current and flame instability. However, by careful selection of fuel gas flow rates these effects may be minimized. Table 2 gives the flow rates appropriate under isobaric and flow-programmed conditions.

Procedure

Field tests were carried out by using the LSD reagent followed by the cocaine reagent. Samples (approximately 1 mg) of the material to be tested were placed on the center of filter papers and one drop of the appropriate reagent was added.

All standard solutions of the local anesthetics were prepared as the hydrochlorides but analyzed as free bases. Solutions for TLC were prepared in ethanol (approximately 10 g/litre) and 2-μl aliquots spotted on to the plate. The tanks were equilibrated for approx-

TABLE 2—*Gas chromatography flow rates.*

Gas	Isobaric Analysis, ml/min	Flow-Programmed Analysis, ml/min	Programming Rate, ml/min^2
Nitrogen (carrier)	30	13 to 150	11
Hydrogen	30	80	...
Air	450	500	...

imately ½ h before use. The solvent was allowed to fully ascend the plate, after which the plates were dried before irradiation and spraying. It is essential to allow the ammonia to evaporate in a fume hood for 5 min or else strong reactions are not obtained with the spray reagent.

Standard solutions of the anesthetics for GC were prepared in ethanol (<400 mg/litre) and 5-μl aliquots introduced by on-column injection. For flow-programmed runs the starting flow rate was set with Regulator R1 and the final flow rate with the main supply regulator.

It was necessary to calibrate the flow controller (F1) digital counter against the rate of programming. After the appropriate conditions (Table 2) have been selected, these items need no further adjustment, unless the GC column is changed. At the beginning of each analysis, Valve V1 was opened to initiate the program, V2, V3, and V4 being closed.

On completion of the run, Valve V1 was closed and V3 and V4 were opened. Column pressure dropped to zero and was returned to the preset starting pressure by closing V3 and V4 and then opening Valve V2. The chromatograph was ready for further use after an equilibration time of 3 min when V2 was closed and the cycle repeated.

Sample Analysis

It is essential that samples taken for analysis are representative of the whole. If no visual evidence would be destroyed, the whole sample should be homogenized mechanically before subsampling. Subsamples should then be ground in a mortar and solutions for TLC or GLC analysis prepared in a manner analogous to that for the standard solutions.

Results

Results of the field tests are given in Table 3. A positive response to these reagents is by no means specific to the local anesthetics and these tests are only for screening purposes. A "positive" response to the LSD reagent is always instantaneous, whereas with the cocaine test response time can extend over several seconds or longer. The variation with the different compounds is itself of diagnostic value and is therefore included in the table.

TABLE 3—*Field test reactions with local anesthetics.*

Compound	Response to LSD Reagent [17]	Response to Cocaine Reagent [5]	
		Less than 5 s	5 to 60 s
Amylocaine	N/C[a]	pale blue	...
Benzocaine	orange	N/C	N/C
Prilocaine	N/C	N/C	N/C
Lignocaine	N/C	pale blue	...
Piperocaine	N/C	blue, instant	...
Tropacocaine	N/C	pale blue	...
Procaine	orange	N/C	N/C
Mepivacaine	N/C	pale blue	...
Tetracaine	orange	blue, instant	...
Cocaine	N/C	blue, instant	...
Bupivacaine	N/C	blue, instant	...
Proxymetacaine	orange	N/C	pale blue
Butacaine	orange	blue, instant	...
Cinchocaine	N/C	blue, instant	...
Cyclomethycaine	N/C	blue, instant	...

[a] N/C = no color.

Results of the TLC analysis are given in Table 4. While only two compounds were detected under irradiation at 254 nm, all were visible under the longer wavelength. All compounds gave a response to the spray reagent and although the colors varied from gray to almost black these variations are of little analytical value. Retention times relative to cocaine under isobaric and flow-programmed GC conditions are given in Table 5. Retention indexes for isobaric conditions are also given. The absolute retention time of cocaine is much shorter under flow-programmed conditions, and even the compound retained longest (cyclomethycaine) elutes in 37 min compared with 93 min under isobaric conditions. Adequate separation of the compounds that were resolved isobarically is still

TABLE 4—*Thin-layer chromatographic characteristics of local anesthetics.*

Compound	Retention R_f		Ultraviolet Irradiation		Reaction to Spray
	Solvent 1	Solvent 2	254 nm	366 nm	
Amylocaine	0.70	0.87	−	+	+
Benzocaine	0.79	0.81	−	+	+
Prilocaine	0.74	0.85	−	+	+
Lignocaine	0.76	0.85	−	+	+
Piperocaine	0.51	0.86	−	+	+
Tropacocaine	0.30	0.86	−	+	+
Procaine	0.57	0.80	−	+	+
Mepivacaine	0.71	0.75	−	+	+
Tetracaine	0.56	0.79	−	+	+
Cocaine	0.63	0.83	−	+	+
Bupivacaine	0.79	0.83	−	+	+
Proxymetacaine	0.63	0.83	+	+	+
Butacaine	0.63	0.84	−	+	+
Cinchocaine	0.60	0.83	+	+	+
Cyclomethycaine	0.51	0.83	−	+	+

TABLE 5—*Gas chromatographic retention data of local anesthetics.*

Compound	Peak Number (Figs. 2 and 3)	Isobaric Run		Flow-Programmed Run
		Relative Retention[a]	Retention Index × 10^3	Relative Retention[b]
Amylocaine	1	0.10	1.81	0.24
Benzocaine	2	0.13	1.93	0.28
Prilocaine	3	0.25	2.16	0.43
Lignocaine	4	0.28	2.20	0.46
Piperocaine	5	0.36	2.28	0.54
Tropacocaine	6	0.36	2.28	0.54
Procaine	7	0.54	2.41	0.69
Mepivacaine	8	0.58	2.43	0.72
Tetracaine	9	0.92	2.57	0.95
Cocaine	10	1.00	2.60	1.00
Bupivacaine	10	1.00	2.60	1.00
Proxymetacaine	...	1.31	2.68	1.18
Butacaine	11	1.94	2.79	1.52
Cinchocaine	12	4.48	3.01	2.83
Cyclomethycaine	13	5.14	3.10	3.74

[a] Absolute retention time of cocaine, 18 min.
[b] Absolute retention time of cocaine, 10 min.

maintained under these conditions despite the substantial reduction in retention time. Chromatograms of runs under the two sets of conditions are shown in Figs. 2 (isobaric) and 3 (flow programmed).

The stability of standard solutions of cocaine hydrochloride (200 mg/litre) was established by analyzing several such solutions of various ages (twelve months, seven months, three months, two weeks, and fresh) at the same time. The standard deviation of the results was 2.4 mg/litre. A comparison of the quantitative results obtained by using cocaine free base and cocaine hydrochloride showed that conversion of the hydrochloride to the base on injection was quantitative. Seven replicate analyses of the two solutions (each 200 mg/litre) gave a standard deviation of 3.4 mg/litre for the hydrochloride and 4.9 mg/litre for the base with a mean conversion of 100.5%.

Solutions containing cocaine in various amounts (20 to 200 mg/litre) to simulate the range of purities (and hence concentrations) encountered in seizures were each analyzed

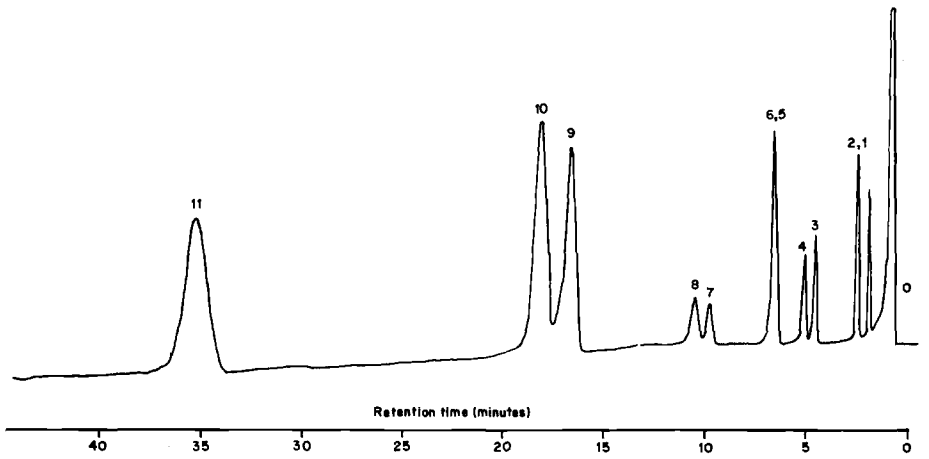


FIG. 2—*Chromatogram of local anesthetics eluted within 40 min with 3% OV-17 column at 220°C under constant carrier gas flow rate. See Table 5 for key to peak numbers.*

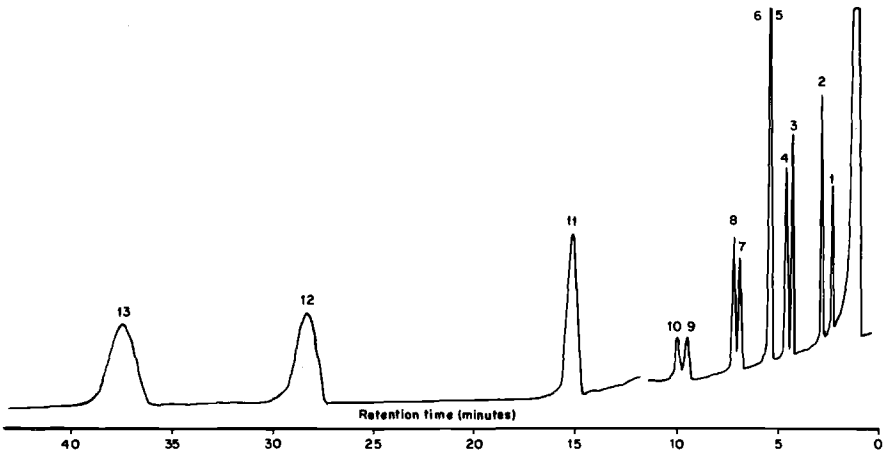


FIG. 3—*Chromatogram of local anesthetics eluted within 40 min with 3% OV-17 column at 200°C under carrier gas flow-programming conditions. See Table 5 for key to peak numbers.*

TABLE 6—*Percentage purity of cocaine seizures by GLC.*

Sample Reference	Percentage Purity	
	By Isobaric GLC	By Flow-Programmed GLC
A	20.0	21.0
B	46.0	45.5
C	54.0	52.5
D	57.0	57.5
E	72.0	71.5
F	101	101

five times. A linear detector response was obtained under both isobaric and flow-programmed conditions. Samples of cocaine seizures were examined in triplicate by both methods and the mean percentage purity was determined by using the appropriate calibration curve. A comparison of the values obtained is given in Table 6, which shows that equally satisfactory data were obtained with each of the two methods.

Summary

The application of field tests and chromatography to the detection of cocaine and some other local anesthetics that have been used to adulterate cocaine is described. Initial screening of samples by field tests, followed by concurrent TLC and GC, enables rapid identification of these compounds to be achieved. In particular, the use of flow-programmed GC shortens the time for analysis compared with conventional GC and requires negligible equilibration time between consecutive runs [*12*]. The method gives reliable quantitative data.

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