$$w = (f_1 w_1 + f_2 w_2 + \ldots f_n w_n) \quad \text{(Eq. 9)}$$

where f_1 is the weight fraction of particles of weight, w_1, *etc.* For spherical particles:

$$w = \frac{\pi\rho}{6} \sum_1^n f_i d_i^3 \quad \text{(Eq. 10)}$$

In the case where particles are the same size, Eq. 10 becomes:

$$w = \frac{\pi\rho d^3}{6} \quad \text{(Eq. 11)}$$

For Eqs. 10 and 11 to be equivalent:

$$d^3 = \sum_1^n f_i d_i^3 \quad \text{(Eq. 12)}$$

Substituting the result from Eq. 8 into Eq. 12 gives $d = 96.2$ μm. Hence, the particle-size distribution given in the example, which was determined by trial and error, is equivalent to the calculated value of $d_{(R)}$ of 96 μm. This distribution is typical of the kind of result encountered in practice. Use of such a particle-size distribution for all active ingredients would allow a safety margin for ingredients *P* and *Q* but, in the case of *R*, would necessitate achieving a truly random mixture to fulfill the desired tolerance range of ±10%.

In practice, a random mix is not always achieved and it may be desirable to introduce an additional safety margin for the lowest concentration drug, *R*. Hersey *et al.* (1) did this in effect by setting the calculated effective mean particle-size limit for *R* as the maximum particle-size limit for the mixture. Alternatively, the coefficient of variation used in Eq. 7 could be set at a lower value than that corresponding to the specified tolerance range of ±10%. For example, instead of 3.333%, a C_v value of 2.5% could be used which would give $d_{(R)}$ from Eq. 7 equal to 79 μm. An equivalent particle-size distribution corresponding to this value of $d_{(R)}$ would contain a considerable fraction above the proposed maximum limit of 96 μm (1) while still incorporating a safety margin to allow for the occurrence of nonrandomized mixing.

In conclusion, converting the particle-size limit into an equivalent particle-size distribution increases the utility of the calculations and provides a more convenient guideline in the practical situation. Additionally, a particle-size distribution of a drug obtained on recrystallization or precipitation or after milling can be tested for its suitability with regard to content uniformity by evaluating $\Sigma f_i d_i^3$ and comparing this value with the value of d^3 derived from Eq. 2 or 7.

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M. C. R. Johnson
Upjohn Limited
Crawley, Sussex
United Kingdom

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Definitive GLC Method of Identifying Cocaine

Keyphrases □ Cocaine—definitive GLC identification □ Trimethylanilinium hydroxide—on-column methylation of cocaine, GLC identification □ GLC—identification, cocaine

To the Editor:

The identification of underivatized cocaine by GLC can be misinterpreted and erroneously reported as pentazocine, levorphanol, or methaqualone when using programmed or isothermal temperatures on 7% OV-17[1]. TLC can also pose problems and lead to the report of a false positive for methadone instead of cocaine (1). Many laboratories are combining mass spectrometry with GLC to provide a more definitive instrumental method for identifying drugs such as cocaine (2); however, many laboratories cannot afford a mass spectrometer and, therefore, more definitive GLC methods of analysis are desirable.

In view of these problems encountered when employing GLC or TLC as a means of identifying cocaine, we wish to report a novel, definitive GLC method of identifying cocaine *via* an on-column GLC reaction under methylation reaction conditions that is applicable to confirming the presence of cocaine in various legitimate and illegitimate dosage forms. In our laboratory we have routinely used trimethylanilinium hydroxide in methanol as a methylating reagent for GLC analysis of anticonvulsant drugs in body fluids (3, 4). We anticipated that this methylating reagent would have an interesting on-column

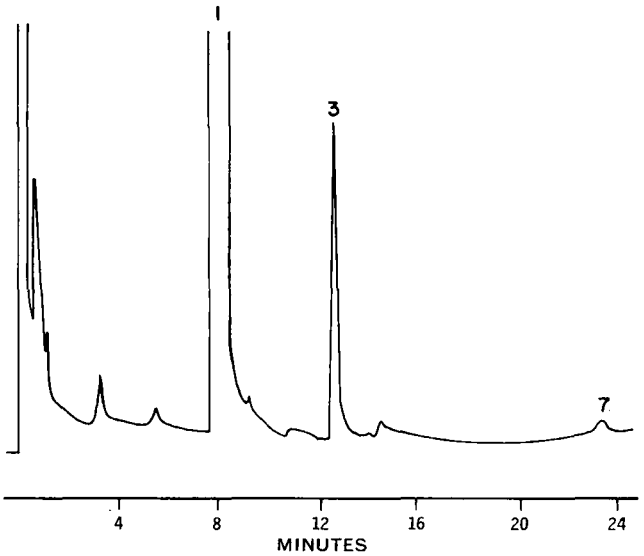

Figure 1—*Characteristic chromatogram representing an on-column reaction of cocaine (1.5 μg) and trimethylanilinium hydroxide (no time lapse after adding the methylating reagent to cocaine). See Table I for identification of the peaks.*

[1] In our laboratory, these drugs have retention times similar to cocaine under programmed and isothermal conditions and are extracted concurrently with cocaine at basic pH.

Table I—Relative GLC Retention Times of Cocaine and Some Commonly Abused Drugs after On-Column Reaction[a]

Drug	R_t, min
Cocaine[b,c]	Peak 1 (8.0), *N,N*-dimethylaniline Peak 2 (10.4), possible Hoffman elimination product Peak 3 (12.5), ecgonidine methyl ester Peak 4 (13.9), under investigation Peak 5 (14.2), ecgonine methyl ester (under investigation) Peak 6 (17.2), under investigation Peak 7 (23.3), cocaine
Amphetamine	8.3
Benzocaine	15.7, 16.8, 17.6, 18.2
Codeine	27.5
Heroin	24.1, 27.2
Levorphanol	21.4
Lidocaine	16.6, 18.5
Methaqualone	23.2
Morphine	27.5
Pentazocine	21.9
Phencyclidine	18.2
Phenobarbital	12.8, 14.3, 14.5, 18.4
Procaine	17.5, 20.8, 21.7, 23.1
Quinine	34.0, 37.0
Secobarbital	15.4

[a] *Under programmed temperature* GLC conditions. [b] The number of chromatographic peaks seen after on-column reaction depends on the concentration of cocaine and the time lapse between addition of the methylating reagent and its on-column injection (see Figs. 1–3). [c] Similar results can be obtained on 3% OV-17 by reducing the nitrogen flow rate from 60 to 30 ml/min and changing the programmed column conditions from 50–250° (10°/min) to 50–250° (8°/min).

reaction with an unusual bicyclic diester tertiary amine structure such as cocaine, through possible ester cleavage and methylation, as well as serve as a definitive confirmatory method by converting it into one or more identifiable derivatives.

All GLC injections were made on a dual-channel instrument[2] equipped with four hydrogen flame detectors. The 1.83-m (6-ft) U-shaped glass columns (2 mm i.d.) were packed with 7% OV-17 on 80–100-mesh Chromosorb W[3]. Operating temperatures were: injector port, 275°; column (isothermal), 250°; column, 50–250° (programmed at 10°/min); and detector, 275°. Flow rates (milliliters per minute) were: nitrogen, 60; air, 300; and hydrogen, 40. Instrumental attenuation was 8×10^{-10}. Under these conditions, reference standard underivatized cocaine had retention times of 23.3 and 2.9 min under programmed and isothermal column temperatures, respectively.

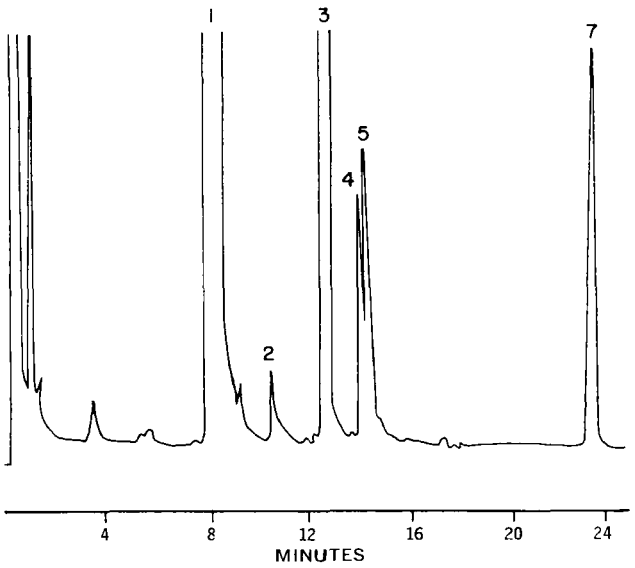

Figure 2—*Chromatogram representing an on-column reaction of cocaine (21 μg) and trimethylanilinium hydroxide (no time lapse after adding the methylating reagent to cocaine). See Table I for identification of the peaks.*

The reaction of cocaine with trimethylanilinium hydroxide solution was initially studied by adding 50 μl of a 2 *M* methanolic trimethylanilinium hydroxide solution[4] (3) to 0.15 mg of cocaine and immediately injecting 0.5 μl (1.5 μg) into the chromatograph under programmed temperature conditions. The resulting chromatogram is illustrated in Fig. 1. Peak 1 is *N,N*-dimethylaniline, a product derived from trimethylanilinium hydroxide during the methylation reaction.

Utilizing a preparative gas chromatograph, we trapped peak 3 and obtained its mass spectrum. Peak 3 was identified as ecgonidine methyl ester (molecular ion, *m/e* 181; base peak, *m/e* 152). Its mass spectrum is identical to the corresponding methylated and trapped reference standard ecgonidine[5]. Ecgonidine methyl ester (peak 3) appeared to be the most characteristic product of on-column reaction between cocaine and trimethylanilinium hydroxide and was observed at all concentrations regardless of the length of time elapsing between addition of the methylating reagent and its on-column injection (peak 3 in Figs. 1–3).

Figure 2 is a representative chromatogram depicting the cocaine–trimethylanilinium hydroxide reaction using a larger amount of cocaine (21 μg), in which case the cocaine–trimethylanilinium hydroxide solution was injected immediately after adding the methylating reagent to cocaine. Mass spectral analysis of peak 2 indicates it to be a potential double Hoffman elimination product, although its structural determination is inconclusive at this time.

Figure 3 represents the chromatogram of the same cocaine–trimethylanilinium hydroxide solution after standing at room temperature for 24 hr. An increase in the concentration of peak 2 (possible Hoffman elimination product) occurred when cocaine was allowed to stand in the alkaline trimethylanilinium hydroxide solution for 1–24 hr (compare Figs. 2 and 3). Peaks 4 and 6 are presently under investigation, and peak 7 is cocaine.

Based on preliminary mass spectral analysis, peak 5 appears to be the ecgonine methyl ester derivative (molecular ion, *m/e* 199; base peak, *m/e* 152). Therefore, to minimize the number of products formed, in-

[2] Varian 2100.

[3] OV-17 and Chromosorb W were obtained from Applied Science Laboratories, Inc., State College, Pa. The 7% OV-17 was prepared in our laboratory using conventional methods (2.1 g OV-17/30 g Chromosorb W).

[4] Trimethylanilinium hydroxide (0.1 *M* in methanol) (trimethylphenylammonium hydroxide) was obtained from Eastman Kodak Co., Eastman Organic Chemicals Division. Trimethylanilinium hydroxide (2 *M* in methanol) was prepared in our laboratory by concentrating 100 ml of 0.1 *M* reagent to 5 ml.

[5] K & K Laboratories, Inc., Plainview, N.Y.

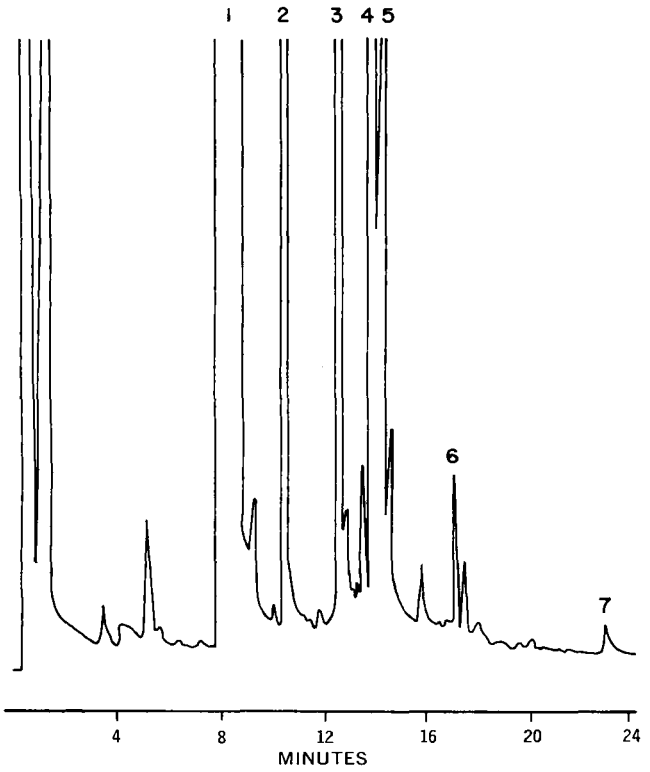

Figure 3—*Chromatogram representing an on-column reaction of cocaine (21 μg) and trimethylanilinium hydroxide (24-hr time lapse after adding the methylating reagent to cocaine). See Table I for identification of the peaks.*

jection of the solution should be performed immediately after adding the methylating reagent to the suspected cocaine residue.

We also wish to report the relative GLC retention times of other commonly abused drugs frequently extracted at alkaline pH ranges (pH 8–10) which could possibly interfere with the confirmation of cocaine by this method. These values are reported in Table I and represent the relative retention times of the product(s) produced using the same on-column reaction conditions performed with cocaine. Of the drugs examined, phenobarbital is the only one that could possibly interfere with the characteristic ecgonidine methyl ester peak (peak 3) under programmed temperature conditions. However, there is no interference between cocaine and phenobarbital when underivatized cocaine is analyzed under isothermal temperature conditions.

To test this method further, we analyzed a simulated street sample containing 6% cocaine hydrochloride, 19% quinine, and 75% dextrose. This analysis was carried out by performing a conventional alkaline extraction (pH 10) of 2.7 mg of the sample with chloroform–isopropanol (3:1), evaporation of the solvent, addition of 50 μl of trimethylanilinium hydroxide to the residue, and immediate injection of 1 μl. The resultant chromatogram resembled Fig. 1, with no interference from quinine.

In summary, we believe this method will be of value as a definitive confirmatory screening test for cocaine after first tentatively identifying underivatized cocaine using isothermal or programmed GLC temperature conditions.

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Richard H. Hammer ˣ
James L. Templeton
H. L. Panzik

Department of Pharmaceutical Chemistry
College of Pharmacy
University of Florida
Gainesville, FL 32610

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ˣ To whom inquiries should be directed.