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Sampling Errors in the Determination of Cocaine in Seized Drugs

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ABSTRACT: Experiments in which 28-g (1-oz) seizures of cocaine plus diluent (mannitol, inositol) were ground in a mortar for only a few minutes before removal of 20-mg portions for assay (by gas chromatography using bupivicaine as internal standard) showed the standard deviation of the sampling operation to be several times larger than the standard deviation of the analytical operations. Measurement of the particle size distribution of ground mixtures allowed estimation of the weight of sample required to lower the sampling standard deviation to any specified level. The sample weight required was shown to depend in a predictable way on the percentage of cocaine present in the material.

KEYWORDS: toxicology, cocaine, sampling

The analysis for legal action of seized illicit drugs such as cocaine is subject to several sources of uncertainty. These include sampling, preliminary treatment of the sampled material, the analytical operation itself, and evaluation of results. Although each of these steps must be considered if the overall error is to be kept small, attention is usually concentrated on the preliminary and analytical steps and little or none on the sampling operations. This neglect can lead to serious errors when the physical form of the substance used as a diluent differs significantly from that of the original drug. In the case of cocaine, seizures often contain hard particles or lumps having high concentrations of the drug, mixed with finely powdered diluents such as mannitol or inositol. Such mixtures clearly require homogenization before sampling for analysis. The usual procedure is to grind the entire seizure in a mortar and pestle until the material has a uniform color and homogeneous appearance.

The purpose of this paper is to show that for this type of mixture the homogenization operation must be monitored closely to avoid introduction of significant uncertainty in the analytical result.

Experimental Procedure

Analytical Procedure

Analyses for cocaine were performed by gas chromatography on a Hewlett-Packard Model 5730A, using a 1.8-m (6-ft) column packed with 3% OV-101 on Gaschrom Q (Chromographic Specialties, Brockville, Ontario) at 210°C. Internal standard solutions were bupivicaine (Winthrop, Lot 1E099) in ethanol (1 mg per millilitre). Calibration curves were prepared by

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injecting a series of standards containing 0.5, 1, 1.5, and 2.0 mg of cocaine hydrochloride (BDH, Lot 83973) per millilitre of internal standard solution. Approximate retention times under the conditions used were 4 min for cocaine and 5.5 min for bupivicaine. Each standard was injected two or three times. Standard curves were drawn by plotting the ratio of peak areas of cocaine to bupivicaine against the concentration of cocaine hydrochloride.

Solutions of unknown material to be analyzed for cocaine were prepared by weighing about 20 mg of the ground material into a tapered 15-mL glass tube and dissolving the material in sufficient internal standard solution to give a signal on the gas chromatograph that fell within the calibration range. From 4 to 10 mL was typically required. The concentration of cocaine hydrochloride present in the unknown material was then read directly from the calibration graph.

Procedure for Grinding and Sieving

All samples of seized material for this study were approximately 28 g (1 oz). Samples to be analyzed were transferred completely into a porcelain or borosilicate glass mortar and ground by hand for varying times. For the sampling studies seven or eight portions of 100 to 200 mg were taken from evenly spaced locations on the surface of the material. From each of these samples, test portions of about 20 mg were taken. No special mixing of the grab samples prior to subsampling was carried out.

For the sieving operations, 76-mm (3-in.) stainless steel sieves were stacked, from top to bottom, in the order 425, 180, 150, 106, 90, 75, and 45 μm (40, 80, 100, 140, 170, 200, and 325 mesh). The sample to be studied was placed in the top sieve, and the stack shaken for approximately 10 min in a Tyler Model RX-24 portable sieve shaker. The fractions were then transferred to plastic cups and weighed, and the percentage of cocaine in each fraction was determined by analysis of single samples.

The relative contributions of the sampling and measurement steps to the overall analytical error were evaluated by the following procedure. A solution of one test portion was injected ten times to determine the reproducibility of the measurement operation, that is, the standard deviation of the analytical method s_a. Then duplicate injections of each of the seven remaining samples were made. The values for the eight samples (the average obtained from ten injections of one and the averages of the two injections from each of the remaining seven) were used to determine the overall standard deviation s_o.

The effect of particle size on the sampling variance was also assessed. A 28-g (1-oz) seizure was placed in a mortar, and eight 200- to 300-mg samples were collected as previously described. Then the seizure was ground until all particles passed a 45-μm (325-mesh) screen. The fine powder was placed in a polyethylene jar large enough to be only about a quarter filled and the jar tumbled end over end for 3 h. The mixed material was returned to the mortar and eight additional samples collected. The 16 samples were then subsampled and analyzed as before. A 20-mg portion of a paste-like material that formed on the pestle midway through the grinding process (but disappeared in the later stages) was also collected and analyzed.

Results and Discussion

In the analytical measurement step, analysts typically monitor procedures to minimize both random and systematic errors. For example, many workers routinely perform multiple injections of the same standard and sample in a gas chromatographic measurement, a time-consuming procedure. The time and effort spent on increasing the precision and accuracy of the analytical technique are of little avail, however, if the portion analyzed is not accurately representative of the material under study.

When sampling for chemical analysis, for random errors the overall standard deviation s_o is related to the standard deviation for the sampling operation s_s and to that for the remaining

analytical operations s_a by the expression $s_o^2 = s_a^2 + s_s^2$. If the sampling uncertainty (standard deviation) is greater than about three times the analytical uncertainty, further reduction in the analytical uncertainty is of little importance unless the sampling uncertainty is correspondingly reduced.

Results of the determination of the standard deviations of the separate operations of sampling and analytical measurement are given in Table 1. Exhibit A shows a standard deviation from sampling that is ten times, while Exhibit B shows one of six times, that caused by the analytical measurements. The analytical measurement standard deviations for both experiments are comparable.

If improvement in the precision of the overall results is sought, attention must clearly be concentrated on sampling. One approach to reduction of the sampling uncertainty in this system would be to obtain a more representative composition in the test portion simply by collecting larger portions. Larger volumes of solvent could then give final concentrations of solution for injection in the same range as before. A larger sample means a greater probability of obtaining a more nearly representative composition in the test portion. An alternative approach is to grind the sample so as to reduce the particle size, thereby providing more particles in the test portion. The effect of time of grinding on the analytical results is given in Table 2.

A way to predict quantitatively the effect of particle size reduction and sample size on the sampling uncertainty is discussed later. Qualitatively, as grinding time is lengthened, the standard deviation of an analytical set can be expected to decrease as a result of decreased sampling uncertainty with increased number of particles and perhaps better mixing. This is seen to occur in one sample in Table 2, but not in the other. The large overall variance in both cases, even after 9 min of grinding, indicates that particle size reduction is still insufficient to reduce the sampling uncertainty.

A factor of equal or greater importance is the range in values of the average percentage of cocaine. Although the range in percentages is within that expected at the 95% confidence level, it is larger than would be expected on the basis of analytical precision. This variability is almost all a result of sampling, and the extent of its reduction with grinding depends on the nature of the individual sample. In Table 2 the standard deviation decreases with longer grinding times for Exhibit C but is relatively unaffected for Exhibit D. We conclude that even

TABLE 1—*Contribution to the overall standard deviation in cocaine analyses from sampling and measurements steps.*

Exhibit	Cocaine · Hydrochloride, Average %[a]	Overall Standard Deviation, s_o	Standard Deviation Analysis, s_a	Standard Deviation Sampling, s_s
A	46.9	1.05	0.10	1.04
B	17.7	0.70	0.11	0.69

[a]Average of eight values.

TABLE 2—*Percentage of cocaine (free base) and standard deviation as function of grinding time.*

	Grinding Time, min				Grinding Time, min		
Exhibit C	2.5	5	7.5	Exhibit D	3	6	9
Cocaine, average %[a]	27.2	29.7	30.0	cocaine, average %[a]	34.8	33.0	31.8
Standard deviation	2.5	2.1	1.6	standard deviation	1.3	1.5	1.4

[a]Average of values from seven independent samples.

with 9 min of grinding the particle size of Exhibit D is still too large to provide enough particles in a 20-mg test portion for a statistically representative number of each type. An alternative explanation is that particle size reduction was adequate but the particles of cocaine and diluent were poorly mixed, even though grinding in a mortar and pestle is usually considered an effective method of mixing powdered materials.

The largest and smallest size fractions of a sample ground in a mortar and pestle for varying times and then sieved is shown in Table 3. Although 98% of the material passes a 425-μm (40-mesh) screen after 9 min of grinding, 58% is still coarser than 75 μm (200 mesh). Since the larger particles control the heterogeneity of the sampling operation, attention should be paid to reducing their size. In cocaine-diluent mixtures the larger particles are cocaine rich, as was determined by analysis of the sieve fractions. The overall composition of the unsieved, ground, sample was 68% cocaine. The sample greater than 425 μm (40 mesh) contained 81% cocaine, while the other fractions ranged from 58 to 69%.

To show that sampling error can be reduced to a low level by sufficient particle size reduction and mixing, a 28-g (1-oz) drug seizure was first sampled as received and then after grinding until all the material passed a 45-μm (325-mesh) sieve and was thoroughly mixed. Results of the two sets of analyses are shown in Table 4. Clearly, the sampling uncertainty can be reduced to the level of that of the analytical measurement when particle size reduction and mixing are adequate.

The paste that formed on the mortar and pestle during grinding of several of the samples tended to be a few percent lower in cocaine than the sample average. This paste may have formed from moisture uptake by the diluents. It sometimes disappeared on continued grinding, and was not a major problem.

Calculation of Effect of Particle Size Reduction

The effect of particle size and sample weight on the uncertainty of the sampling operation for cocaine seizures can be calculated by the method of Benedetti-Pichler [*1,2*]. For a mixture of pure cocaine plus diluent the relative standard deviation (coefficient of variation) in sample

TABLE 3—*Fraction of sample in coarse and fine size ranges as function of grinding time.*

Particle Size		Grinding Time, min		
Mesh	mm	3	6	9
>40	>0.425	0.12	0.07	0.02
<200	<0.076	0.14	0.17	0.42

TABLE 4—*Percentage of cocaine (free base) and standard deviation for sample as-received and ground to finer than 45* μm (325 mesh).

Exhibit E	Cocaine (Free Base), Average %[a]	Standard Deviation	Relative Standard Deviation, ppt
As received	33.6	6.5	193
Ground to 45 μm (325 mesh)	27.57	0.15	5

[a]Average of values from eight independent samples.

composition R in parts per thousand (ppt) can be related to the total number n of the two types of uniformly sized particles by

$$R = \frac{d_1 d_2}{\bar{d}^2} \frac{10^4}{\bar{P}} \sqrt{\frac{p(1-p)}{n}} \quad (1000)$$

where d_1 and d_2 are the densities of cocaine and diluent, $\bar{d}$ is the average density of the mixture, $\bar{P}$ is the average percentage of cocaine, and p and $1 - p$ are fractions of the two kinds of particles. Using 1.25 and 1.49 as the densities of cocaine [3] and mannitol [4], a common diluent, it is possible to calculate the effect of the number of particles taken upon the standard deviation for a perfectly mixed sample. This number can be related to the weight of the sample by assuming a uniform specified particle shape. For the reasonable assumption of spherical particles, the relation between sample weight and n is

$$\text{sample weight} = (4/3)\pi r^3 \bar{d} n$$

where r is the particle radius.

Figure 1 shows the relation predicted between particle size and minimum sample weight required if the relative standard deviation in sampling $s_s/\bar{x}$ is to be held to 10 ppt (1% relative).

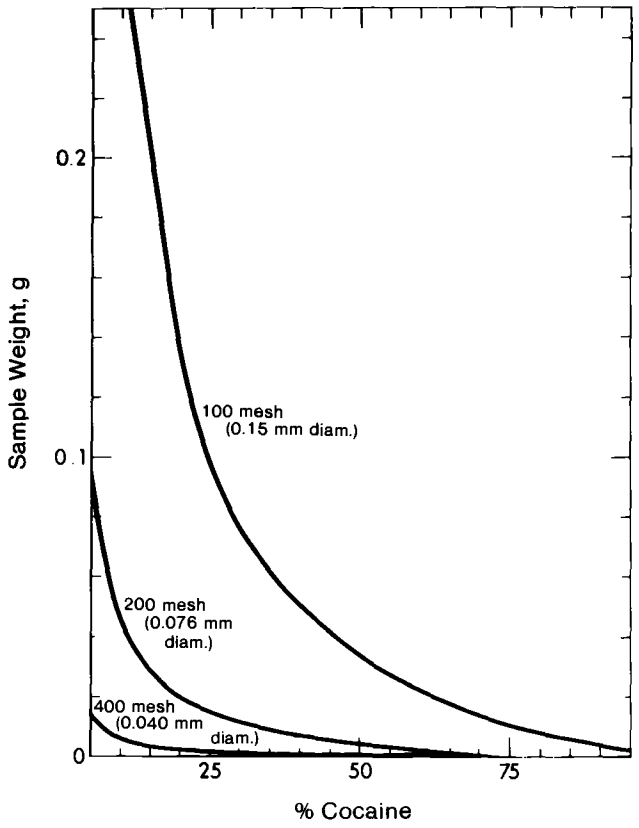

FIG. 1—*Relation between weight of sample required and percentage of cocaine for three particle sizes for a sampling standard deviation of 10 ppt for mixtures of pure cocaine hydrochloride and diluent. (Assumptions: spherical particles of uniform size and complete mixing.)*

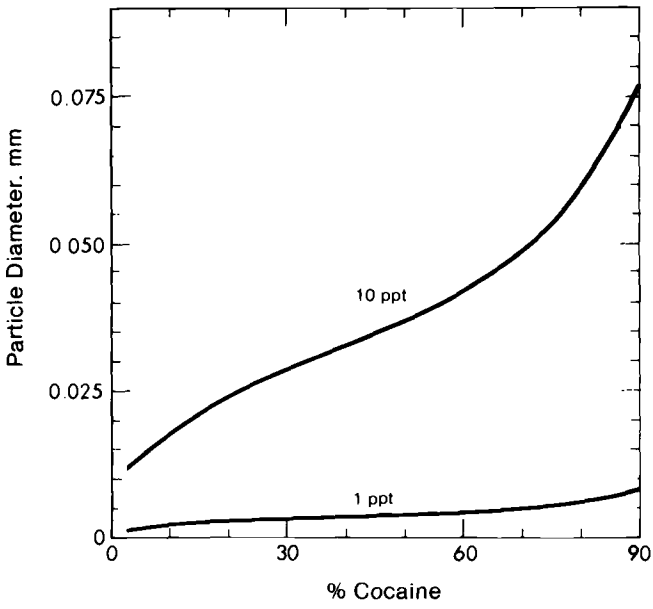

FIG. 2—*Plots of particle diameter against percentage of cocaine for sampling standard deviations of 10 and 1 ppt. (Assumptions as in Fig. 1.)*

Although these calculations are based on uniformly spherical particles, the results are not affected in a major way by assuming other shapes. It is clear that if a test portion of only 20 mg is to be taken for analysis, and the sampling standard deviation is to be held to the level of that of the analysis, the particle size must be of the order of 38 μm (400 mesh) unless the percentage of cocaine is above 20. The analyst can increase the number of particles in the test portion either by increasing the size of the test portion or by reducing the particle size.

If a relative standard deviation of less than 10 ppt is desired for a 20-mg sample, then the particles must be smaller still. Figure 2 shows the relation between particle diameter and percentage of cocaine for two levels of sampling relative standard deviation, 1 and 10 ppt. The lower level can be attained only if the particle diameter is held below about 10 μm, which is unattainable for most cocaine-diluent mixtures in a mortar and pestle without unreasonable effort.

In summary, these results indicate sampling uncertainty to be the major contributing factor to the overall standard deviation in the determination of cocaine in drug seizures when manual grinding is used. Additional refinement of the measurement operation is neither cost- nor time-effective. Injection of more than duplicate portions in the chromatographic step will not significantly improve the results. Rather, much larger test portions should be dissolved and aliquots taken, or the original seizure should be ground to a specified smaller particle size before sampling. Because of the variability in hardness and particle size of street seizures, it is recommended that the material be ground with a mechanized grinder until all passes a preestablished sieve size.

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