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The Homogenization of Illicit Heroin Samples: An Empirical and Statistical Approach

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ABSTRACT: Although methods for sampling and testing illicit heroin are well known, a method for the homogenization of a heroin sample has not been statistically established. This paper reports the conditions for homogenizing granular or powdery illicit heroin samples using a blender. The experimental results and statistical analysis show that the homogenization of illicit heroin containing various concentrations of diamorphine can be achieved after blending for three min.

KEYWORDS: criminalistics, homogenization, heroin, powder, statistics

The analysis of illicit heroin is well established [*1*], and there are several sampling procedures for obtaining a representative sample [*2–4*]. However, method of homogenization of illicit heroin, which is normally received by the laboratory in the form of a granular or powdery substance, has so far not been published although the conditions for the blending and premixing of metal powders have been discussed in detail [*5*].

Homogenization is a very important step in the laboratory quantification of diamorphine in illicit heroin exhibits. This is especially so in cases where the drug traffickers face capital punishment for trafficking in quantities of the drugs in excess of the specified limits. In Singapore under the Misuse of Drugs Act (1985 Edition), trafficking in more than 15 grams of diamorphine is a capital offense. In this paper, the blending of granular or powdery substances is studied using both simulated mixtures containing different amounts of codeine and glucose and illicit heroin exhibits to establish the conditions and time necessary to achieve a homogeneous product. Quantification of codeine in the simulated mixtures and of diamorphine in illicit heroin exhibits are carried out with high performance liquid chromatography (HPLC).

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Materials and Methods

Instrumentation

A Hewlett-Packard 1090 Series L liquid chromatograph equipped with an autoinjector and a filter photometric detector was used in this study. HPLC chromatograms were recorded on a HP 3396A integrator.

HPLC separation was carried out at 40°C on a 200 by 4.6 mm i.d. column packed with Hypersil ODS (5 μm). The mobile phase was methanol/phosphate buffer (1:1) at a flow rate of 1.5 mL/min. The eluent was monitored at 280 nm.

Blending was carried out by either a Kenwood domestic blender or a Waring laboratory blender. Both were used with a one-liter container.

Chemicals

Methanol (HPLC grade) was obtained from J. T. Baker Chemical Co. Diamorphine hydrochloride was obtained from MacFarlan Smith Ltd., UK. Codeine phosphate (BP) was obtained from Sunward Pharmaceutical Pte Ltd, Singapore. Nalorphine hydrobromide was obtained from The Wellcome Foundation Ltd., UK. All other chemicals used were of analytical grade.

Mobile Phase

Phosphate buffer (pH 6.6) was prepared by mixing solutions of potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate, both 0.01 mol/L, in the ratio of 5:2. The buffer was mixed with methanol in the ratio of 13:12 and filtered through a 0.45 μm membrane filter before use.

Analytical Standards

Working solutions of codeine and diamorphine were prepared by dissolving the drug in methanol to give a concentration equivalent to 1.0 mg/mL of free base. A 1.0 mg/mL solution of nalorphine, which was used as the internal standard, was similarly prepared. All solutions were stored under refrigeration.

Homogenization Studies

Different amounts of codeine phosphate and glucose were used to give a codeine concentration (calculated as codeine base) of 4.9, 10, and 20% while maintaining the total weight of the powder at 150 g. The powder was mixed in the Kenwood blender over a period of 12 min. After each minute interval, 16 test samples of about 0.25 g each were removed at random and analyzed by HPLC for the codeine content.

A codeine/glucose mixture containing 10% of codeine was blended in a Waring blender as described above. After each minute interval, 16 test samples were removed and analyzed. A similar mixture was blended for 20 min in the Waring blender and sixteen test samples were removed and analyzed.

Illicit heroin samples used for the studies were obtained from exhibits submitted to the laboratory by the enforcement agency. Only exhibits weighing between 200 to 400 g that could be blended in one portion in the one-liter container were selected. To establish the extent of homogeneity of each exhibit before blending, 16 random test samples of about 0.25 g each were removed before blending from the exhibit and analyzed by HPLC. The rest of the exhibit was then mixed in the Waring blender and 16 samples were

removed and analyzed at one-minute intervals as described for the codeine/glucose mixtures.

Sample Preparation

About 0.25 g of sample (codeine/glucose mixture or heroin exhibit) was accurately weighed and dissolved in a suitable volume of methanol. A 1 mL aliquot was transferred to a stoppered tube containing 1 mL of the internal standard solution. A total of 10 μL of this solution was injected into the HPLC. Quantitative determination was carried out by measuring the peak areas of codeine (or diamorphine) and the internal standard.

Statistical Analysis

Since the coefficient of variation (CV) is a measure of relative variability, it can be regarded as a measure of homogeneity. In our experiment, 16 test samples were withdrawn randomly after each one-minute interval of blending, and the content of codeine was analyzed by HPLC. The estimated % CV is obtained from the results of the 16 test samples as

$$\% \text{ CV} = \frac{\text{Sample Standard Deviation}}{\text{Sample Mean}} \times 100\%$$

A plot of the estimated CV against time is constructed to assess the point at which the CV will level off and approach the value of the limiting CV. The limiting CV is the variation attributable to the analytical technique at the particular codeine concentration. The mixture can be regarded to be sufficiently homogenized if the estimated CV is close to the limiting CV.

To assess whether the difference in homogeneity (or variability) between two sets of samples is statistically different, we consider the conventional variance homogenization test based on the following statistic

$$T_{i,j} = \frac{S_i^2}{S_j^2}$$

where S_i^2 and S_j^2 are the sample variances for sets of samples collected after blending for i and j minutes, respectively.

It is well known that if the sample observations come from a Normal distribution, $(n_i - 1)S_i^2/\sigma_i^2$ and $(n_j - 1)s_j^2/\sigma_j^2$ are distributed as a X^2 distribution with $(n_i - 1)$ and $(n_j - 1)$ degrees of freedom. Since the samples are drawn independently, it follows that if $\sigma_i^2 = \sigma_j^2$, $T_{i,j}$ is distributed as a F-distribution with $(n_i - 1)$ and $(n_j - 1)$ degrees of freedom [6]. Thus, $T_{i,j}$ may be used to assess whether the difference in homogeneity (or variability) between two sets of samples is statistically different, that is, we can test the null hypothesis that $\sigma_i^2 = \sigma_j^2$. In particular, if the limiting CV is reached after k minutes, we consider the statistic $T_{i,k}$ which, under the conditions of our experiments will be distributed as a F-distribution with $(n_i - 1)$ and $(n_j - 1)$ degrees of freedom.

Results and Discussion

Table 1 shows the results derived from a typical homogenization experiment together with mean, standard deviation (SD) and the estimated CV calculated for each time interval. Table 2 summarizes the statistical data obtained from the codeine/glucose mixtures containing different codeine concentrations. A plot of CV against time is given in

TABLE 1—*Typical statistical data of a codeine/glucose mixture (codeine concentration = 4.9%).*

Sample	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min	7 Min	8 Min	9 Min	10 Min	11 Min	12 Min
No. 1	4.277	4.620	4.660	4.874	4.833	4.901	4.914	4.945	4.770	4.886	4.859	4.804
No. 2	4.960	4.751	4.788	4.976	4.813	4.978	4.865	4.960	4.893	4.928	4.890	4.855
No. 3	4.888	4.787	4.624	4.807	4.975	4.874	4.967	4.903	4.879	4.923	4.896	4.896
No. 4	4.674	4.807	4.679	4.925	4.895	5.061	4.915	4.938	4.878	4.980	4.911	4.902
No. 5	5.349	4.759	4.615	4.792	4.869	4.992	4.950	4.982	4.832	4.976	4.946	4.876
No. 6	4.405	4.683	4.676	4.827	4.877	4.983	4.831	4.840	4.749	4.811	4.858	4.890
No. 7	4.442	4.749	4.808	4.928	4.911	5.015	4.795	4.995	4.810	4.963	4.779	4.871
No. 8	4.797	5.106	4.682	4.943	4.902	5.050	4.884	5.044	4.833	4.947	4.942	4.831
No. 9	4.895	4.789	4.796	4.988	4.912	4.940	4.894	4.878	4.865	4.921	4.897	4.793
No. 10	5.026	4.896	4.893	4.894	4.943	4.880	4.982	4.856	4.933	4.956	4.888	4.935
No. 11	4.438	4.893	4.876	4.972	4.962	4.883	4.878	4.965	4.911	4.848	4.935	4.886
No. 12	4.862	4.936	4.598	4.846	4.908	4.967	4.862	4.926	4.880	4.976	4.959	4.836
No. 13	4.857	4.743	4.929	4.918	4.859	4.952	4.947	4.881	4.805	4.844	4,869	4.930
No. 14	5.372	4.848	4.867	4.988	4.826	4.903	4.911	4.895	4.800	4.980	4.927	4.863
No. 15	5.349	5.093	4.904	5.023	4.732	4.951	4.988	4.853	4.841	4.915	4.903	4.906
No. 16	5.546	5.239	4.779	4,853	4.734	4.828	4.980	5.003	4.892	4.882	4.923	4.970
Mean	4.884	4.856	4.761	4.910	4.872	4.947	4.910	4.929	4.848	4.921	4.899	4.878
Standard deviation	.381	.167	.113	.071	.071	.066	.057	.060	.052	.053	.044	.047
% Coeff of variation	7.806	3.432	2.377	1.442	1.457	1.332	1.154	1.220	1.070	1.086	.902	.973

TABLE 2—*Statistical data of codeine/glucose mixtures.*

	4.9% (Kenwood)			10% (Kenwood)			20% (Kenwood)			10% (Waring)		
Time interval	Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %
1 min	4.844	.381	7.806	9.820	.323	3.286	20.092	.566	2.815	9.795	.401	4.092
2 min	4.856	.167	3.432	9.858	.223	2.258	19.511	.360	1.847	10.053	.163	1.619
3 min	4.761	.113	2.377	9.845	.194	1.975	19.819	.317	1.600	9.923	.137	1.382
4 min	4.910	.071	1.442	9.856	.171	1.733	19.987	.271	1.356	10.074	.127	1.265
5 min	4.872	.071	1.457	9.850	.152	1.540	20.057	.207	1.030	10.051	.117	1.160
6 min	4.947	.066	1.332	9.858	.127	1.293	19.799	.173	.875	9.989	.098	.982
7 min	4.910	.057	1.154	9.927	.107	1.077	20.106	.182	.903	9.933	.097	.979
8 min	4.929	.060	1.220	10.151	.107	1.050	20.188	.166	.822	9.818	.094	.960
9 min	4.848	.052	1.070	9.944	.093	.939	20.142	.149	.740	9.816	.092	.938
10 min	4.921	.053	1.086	9.915	.090	.903	19.748	.129	.651	9.945	.088	.888
11 min	4.899	.044	.902	9.919	.089	.893	20.104	.134	.664	9.973	.090	.903
12 min	4.878	.047	.973	9.919	.087	.875	20.169	.127	.627	9.919	.086	.868

Fig. 1. Several interesting points were observed from Table 2 and Fig. 1. First, in all cases the CV decreases rapidly within two minutes of blending, and level off within about five minutes. Second, the higher the codeine content, the smaller is the initial CV (at one minute) and the faster the CV decreases. Third, a domestic blender (Kenwood) is as effective as a laboratory blender (Waring) in producing a homogeneous mixture. However, the laboratory blender produces a homogeneous mixture in a shorter time than the domestic blender.

To determine the limiting CV at each codeine concentration, 16 replicates of HPLC analysis were carried out on one single test sample for each codeine concentration. The CVs were found to be 0.87, 0.76, and 0.54% for 4.9, 10 and 20% codeine, respectively. Comparing these values with those in Table 2, it is observed that the CVs at 12 minutes are only marginally higher than the limiting CVs in all cases. In one study (10% codeine content), the codeine mixture was blended for 20 minutes with a Waring blender. The CV was found to be 0.86%, which is similar to that at 12 minutes. Based on the above observations, it can be concluded that blending with an ordinary domestic blender would produce a homogeneous mixture within about five minutes irrespective of the concentration of codeine present. Further blending does not significantly improve the homogeneity.

Another interesting observation is that in all cases, the means were very close to the expected values (ranging from −2.8% to 1.5% of the expected values) irrespective of the blending time and CV. In the case of the 4.9% codeine mixture, for example, the CV at one minute was 7.8%, yet the mean was 99.5% of the expected value (Table 2). This suggests that in cases where samples cannot be properly homogenized, taking a sufficient number of random test samples would effectively provide a good estimate of the actual mean.

Since the limiting CV is reached within 12 minutes, we consider the statistic $T_{i,12}$. With $n_i = n_{12} = 16$, we compare the value of $T_{i,12}$ with the F-distribution with (15,15) degrees of freedom. Table 3 shows the values of $T_{i,12}$ for the codeine/glucose mixtures. Since the 95th percentile of the F-distribution with (15,15) degrees of freedom is 2.40, we can infer

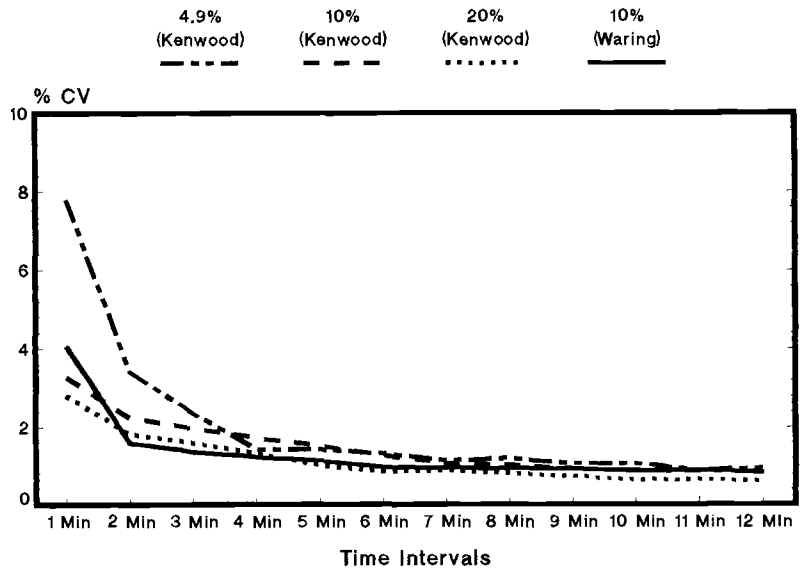

FIG. 1—*Plot of %CV Against Time for Codeine/Glucose Mixtures.*

TABLE 3—*Values of $T_{i,12}$ for codeine/glucose mixtures.*

Time interval	Mixture 1	Mixture 2	Mixture 3	Mixture 4
1 min	65.71	13.78	19.86	21.74
2 min	12.63	6.57	8.04	3.59
3 min	5.78	4.97	6.23	2.54
4 min	2.28	3.86	4.55	2.18
5 min	2.28	3.05	2.66	1.85
6 min	1.97	2.13	1.86	1.30
7 min	1.47	1.51	2.05	1.27
8 min	1.63	1.51	1.71	1.19
9 min	1.22	1.14	1.38	1.14
10 min	1.27	1.07	1.03	1.05
11 min	0.88	1.05	1.11	1.10
12 min	1.00	1.00	1.00	1.00

from Table 3 that at $\alpha = 0.05$ the variability after blending for 6 minutes is not significantly different from that at the limiting CV, that is, after blending for 12 minutes. With the 99th percentile of the F-distribution with (15,15) degrees of freedom being 3.52, the variability after blending for five minutes is not significantly different at $\alpha = 0.01$ from that after blending for 12 minutes. This conclusion is consistent with the plot of CV against time.

It can be concluded from the above observations that a plot of the CV against time can be usefully adopted as an indicator for the degree of homogeneity in illicit heroin analysis and that the minimum blending time required to produce a homogeneous product is 5 minutes. Three illicit heroin exhibits were analyzed before and after blending at 1, 3, 5, 7 and 12 minute time intervals. Table 4 shows the typical results obtained from one

TABLE 4—*Typical statistical data of an illicit heroin exhibit.*

Sample	0 Min	1 Min	3 Min	5 Min	7 Min	12 Min
1	7.564	7.601	7.172	7.308	7.333	7.438
2	7.320	7.356	7.364	7.147	7.359	7.334
3	7.725	7.419	7.287	7.260	7.109	7.385
4	7.384	7.301	7.114	7.091	7.418	7.425
5	6.602	7.340	7.038	7.074	7.360	7.448
6	7.697	6.930	7.249	7.183	7.358	7.416
7	7.237	7.850	7.044	7.126	7.396	7.417
8	7.286	7.282	7.096	7.354	7.385	7.478
9	7.466	7.328	7.090	7.146	7.307	7.125
10	5.331	7.367	7.370	7.356	7.363	7.412
11	7.376	7.286	7.244	7.054	7.368	7.406
12	7.275	7.336	7.113	7.120	7.415	7.332
13	7.569	7.348	7.125	7.103	7.129	7.321
14	7.714	7.888	7.113	7.104	7.381	7.307
15	7.372	7.339	7.177	7.140	7.123	7.174
16	7.314	7.263	7.390	7.160	7.314	7.212
Mean	7.265	7.390	7.187	7.170	7.320	7.352
Standard deviation	.580	.228	.117	.097	.104	.103
Coeff of variation	7.985%	3.083%	1.622%	1.348%	1.416%	1.406%
Difference between highest and lowest values	44.90%	13.82%	5.00%	4.28%	4.34%	4.95%

heroin exhibit while Table 5 summarizes the statistical data for the three cases. The plots of CV against time for the three heroin exhibits are shown in Fig. 2. Table 6 shows the values of $T_{i,12}$ for the three illicit heroin exhibits.

As can be seen from Table 4, the CV can be as high as 8% if no blending was carried out. The difference between the highest and lowest values is about 45% indicating a relatively low degree of homogeneity. After five and 12 minutes of blending, the CV declined to about 1.3 and 1.4%, with the difference between the highest and lowest values being about 4.3 and 4.9%, respectively, indicating a high degree of homogeneity. The values of $T_{i,12}$ show that the variability after blending for three minutes is not significantly different from that after blending for five, seven, and 12 minutes at both $\alpha = 0.05$ and $\alpha = 0.01$. This shorter time was expected because illicit heroin exhibits already have some degree of homogeneity (Table 4) whereas the simulated codeine/glucose mixtures were initially completely heterogeneous.

It is also observed from Table 5 that the mean values obtained for illicit heroin exhibits without blending and after blending for one, three, five, seven, and 12 minutes were all very close to each other in all cases. These results are consistent with the inferences drawn from the codeine/glucose mixture experiments.

TABLE 5—*Statistical data of illicit heroin exhibits.*

Time interval	MEAN			SD			CV (%)		
	Ex 1	Ex 2	Ex 3	Ex 1	Ex 2	Ex 3	Ex 1	Ex 2	Ex 3
0 min	10.08	7.26	3.22	0.69	0.58	0.26	6.86	7.98	8.09
1 min	9.85	7.39	3.24	0.15	0.22	0.03	1.49	3.08	0.83
3 min	9.81	7.18	3.26	0.17	0.11	0.04	1.71	1.62	1.07
5 min	*	7.17	3.14	*	0.09	0.02	*	1.34	0.69
7 min	9.75	7.32	3.06	0.10	0.10	0.02	0.98	1.41	0.75
12 min	9.66	7.35	3.01	0.14	0.10	0.03	1.44	1.40	0.96

*Analysis not done.

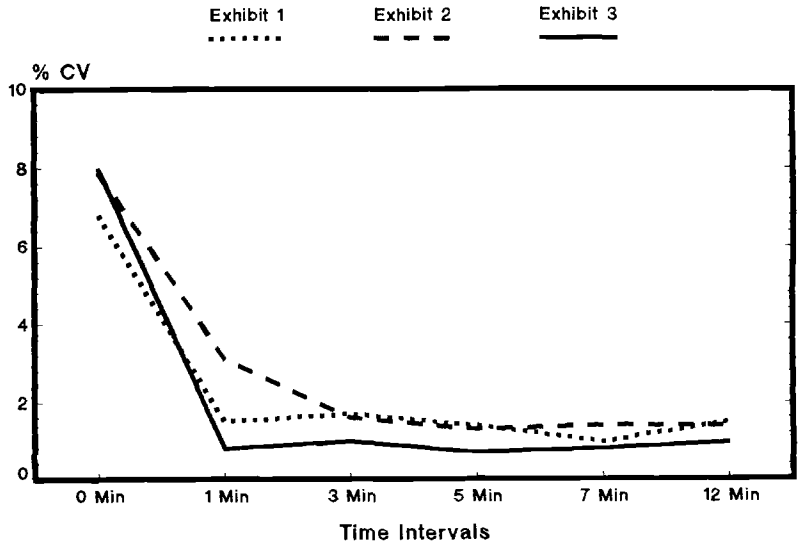

FIG. 2—*Plot of %CV Against Time for Illicit Heroin Exhibits.*

TABLE 6—*Values of $T_{i,12}$ for illicit heroin exhibits.*

Time interval	Exhibit 1	Exhibit 2	Exhibit 3
0 min	24.29	33.64	75.11
1 min	1.15	5.29	1.00
3 min	1.47	1.44	1.78
5 min	*	1.00	0.44
7 min	0.51	1.00	0.44
12 min	1.00	1.00	1.00

*Data not available.

Conclusions

The homogenization experiments demonstrated that the CV may be usefully adopted as a measure of homogeneity in illicit heroin analysis. A plot of CV against time provides useful information regarding the homogeneity of the mixture. The experiments and statistics also demonstrated that:

1. granular or powdery substances can be homogenized after 3 minutes of blending using a domestic blender;
2. regardless of the homogeneity of the exhibit, a sufficient number of random samples can provide an accurate estimate of the content of drug in the exhibit.

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References

[1] Gough, T. A., Ed., *The Analysis of Drugs of Abuse*, John Wiley & Sons Ltd., Chichester, 1991, Chapters 2, 4 and 5.
[2] *Determination of Particle Size Distribution, Part 1. Guide to Powder Sampling*, BS 3406, Part 1, 1986, British Standards Institution.
[3] *Recommended Methods for Testing Heroin*, United Nations, 1986.
[4] Frank, R. S., Hinkley, S. W., and Hoffman, C. G., "Representative Sampling of Drug Seizures in Multiple Containers," *Journal of Forensic Sciences*, Vol. 36, No. 2, March 1991, pp. 350–357.
[5] Dotter, R. T., "Blending and Premixing of Metal Powders," *Powder Metallurgy, Metals Handbook*, Ninth Edition, American Society for Metals, Vol. 7, pp. 186–189.
[6] Hoel, P. G., Introduction to Mathematical Statistics, 5th Edition, 1984, John Wiley & Sons.

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