EUROPEAN NETWORK OF FORENSIC SCIENCE INSTITUTES - DRUGS WORKING GROUP

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Guidelines on Sampling of Illicit Drugs for Quantitative Analysis

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Guidelines on Sampling of Illicit Drugs for Quantitative Analysis

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Preface

In 2001 the European Network of Forensic Science Institutes (ENFSI) Drugs Working Group (DWG) decided to prepare guidelines on representative sampling of illicit drugs for qualitative analysis [1]. The basis of this document was to propose sampling strategies for seizures with large numbers of units, to provide evidence of the presence or the absence of illicit compounds. As a consequence, only a fraction of the total units in a seizure needed to be fully investigated. These guidelines (Guidelines on representative drug sampling) were published in 2003. The qualitative sampling plans in the ENFSI guidelines use probability calculations (e.g. Hypergeometric and/or Bayesian statistics) to answer the basic question 'To a given probability, does the specified proportion of units in a seizure contain a drug (yes or no)'?

Quantitative analysis of a drugs seizure answers the question 'what is the concentration of a drug in a seizure'? Therefore, it is not possible to use the same statistical approach (as recommended for qualitative analysis) to sample for quantitative analysis, because the measured quantity of a drug within a seizure may be heavily influenced by the heterogeneity of that seizure. Furthermore, the qualitative sampling statistical approach when applied to quantitative sampling would not be practicable, as it could generate too many samples for quantitative analysis. Therefore, a different sampling protocol needed to be devised for quantitative analysis.

In 2007 a sub-committee of the ENFSI WG-Drugs was created in order to devise suitable sampling guidelines for the quantitative analysis of different illicit drugs. A prerequisite of these guidelines was that they be practical and economical, as well as adhering to scientifically recognised sampling theories.

Glossary

Analytical sample (m_n)	Last chosen material after homogenisation and mass reduction which will be dissolved for instrumental analysis.
Bulk	The whole amount of material, which has been considered to be associated. In a multiple package seizure the bulk is not visually or by other preliminary tests differentiable.
Constant factor of constitution heterogeneity (IHL)	Constant value, for the given state of the material, that numerically represents its constitution heterogeneity.
Constitution heterogeneity	Refers to the differences in the constitution of the material i.e. how alike or different the individual particles or molecules are. It characterises the variation between individual fragments or particles (for solids) or between individual molecules (for liquids and gases).
Correct sampling	Specific term from Pierre Gy's theory of quantitative sampling process. The principle of 'correct sampling' for all samples (bulk solids, liquids and gases) is based on 2 statements: (1) Every unit in the lot or population has an equal chance of being in the sample. (2) The integrity of the sample is preserved during and after sampling (i.e. the sample stays the same between the time it is taken and the time it is analysed). Samples taken using 'correct sampling' are representative by nature. Correct sampling comprises the whole process, i.e. from the primary sample to analytical sample.

Distribution heterogeneity	Refers to the differences in how the pieces (fragments, particles or molecules) are distributed spatially i.e. how well mixed or segregated the material is due to density, particle size, or other factors.
Fundamental error (FE)	The fundamental error (FE) is the minimum error generated when a sample of a given weight is collected and is influenced by its particle size. The fundamental error is inherent in the compositional distribution of the sample material and cannot be changed without changing the nature of the sample. This error can be reduced either by increasing the mass of the sample or by decreasing the size of the largest particles in it, by comminution.
Herbal cannabis Type I	Bulk material of herbal cannabis which basically contains cannabis buds.
Herbal cannabis Type II	Bulk material of herbal cannabis which consists of different particles (typically buds, leaves, stems and small fragments) and has a broad constitution heterogeneity, for which the incremental sampling of 1 g or 1 bud cannot be applied.
Increment	Randomly chosen portion from the bulk material from which the primary sample is assembled. In this guideline the increment size is fixed to 1 g of powder or 1 tablet or 1 cannabis bud.
Lot mass (m_0)	Net weight of the bulk material.
Mass reduction	Sub-division of the bulk material to obtain a sample size suitable for analysis.
Maximum heterogeneity	Heterogeneity that corresponds to the 90% cumulative percentage (i.e. 90% of the analysed seizures had a lower heterogeneity).

Mineralogic factor (*c)*	Mineralogic factor or composition factor, related to a density in g/cm^3. The mineralogic factor *c* is defined as the maximum heterogeneity generated by the constituent (analyte) of interest in the lot. This maximum is reached when the constituent of interest forms two fractions, one containing the constituent of interest and the other containing none of that constituent.
Nominal particle size (d)	The nominal particle size in the sample, equivalent to the maximum particle size in the lot to be sampled. In practice, d is taken to be the screen size that retains 5% of the lot being sampled. Diameter of the largest particles in the illicit drugs we studied was deduced by experiment.
Primary sample (m_1)	Chosen material obtained from the bulk by correct incremental sampling.
Qualitative sampling	Overall sampling process to clarify whether the target compound is present in at least a certain percentage of the individual items that make up the bulk material and to a required confidence level.
Quantitative sampling	Overall sampling process to determine the average concentration of the target compound in the bulk material.
Range chart (R-chart)	Form of graphical quality control, where the range of results originating from individual samplings is compared to a given warning limit or action limit. The chart can be used to evaluate whether the material has typical heterogeneity or not and if the sampling process was carried out in a repeatable way. The range is the difference between the minimum and maximum value, thus in case of duplicate sampling the range is equal to the difference between the two results.

Relative standard deviation chart(S-chart)	Form of graphical quality control, where the relative standard deviation of multiple results from multiple samplings is recorded on an S-chart to a given warning limit or action limit. The chart can be used to evaluate whether the material has typical heterogeneity or not and if the sampling process was carried out in a repeatable way.
RSD	Relative standard deviation.
SAD (split absolute difference) method	Method for the control of incremental sampling. Increments comprising the sample are allocated to either of two equal sized ‘splits’, which are prepared and analysed separately and the difference between the results is evaluated.
Typical heterogeneity	Calculated value as the square root of the average variance of experimental concentrations.

1 Recommendations

The recommendations in these guidelines are based on the results of drug homogeneity studies [2] and particle size investigations [3]. Using that information a general sampling plan (depicted in the form of two flow-charts) has been devised that could be applied to the quantitative instrumental analysis of the most common illicit drugs: namely heroin, cocaine, amphetamine, cannabis resin, MDMA tablets and herbal cannabis in 'bud' form (Type I). Other more heterogeneous forms of cannabis (Type II) were found to require alternative, more traditional sampling methods (e.g. coning and quartering).

Where practical the homogenisation of the whole material is the preferred sampling process. However, where either the total amount of the seized material or the number of multiple packages involved excludes this preferred option, then an incremental sampling process (taking random increments from the whole seizure and combining them to produce a primary sample(s)) should be applied.

Assuming a laboratory wishes to quantitatively analyse a seizure of one of the named powdered drugs or cannabis resin with a 'typical' heterogeneity, a primary sample of 15 × 1 g increments is generally appropriate. The appropriate primary sample for MDMA tablets is 20 tablets, while for herbal cannabis in bud form (Type I) 50 buds was found to be appropriate.

For a suitably homogenised primary sample of most common powdered drugs an analytical sample size of between 20 and 35 mg is generally appropriate, while for herbal cannabis the analytical sample size is 200 mg.

In general, based on our findings, one incremental sampling of a seizure to produce primary sample should be sufficient. However, where the likelihood of atypical heterogeneity is high or legal requirements governing laboratory procedure are strict then we recommend duplicate or multiple incremental sampling. Results of independent samplings can be compared to demonstrate whether or not a particular seized material has a 'typical' heterogeneity and that the sampling procedure applied has resulted in a 'correct sample'. The setting up of suitable control charts for quality control purposes is also recommended.

2 Introduction

The overall aim of these guidelines was to devise suitable sampling plans and sample preparations for the quantitative instrumental analysis of the most common illicit drugs, based on theoretical background from the literature and our experimental data.

It was recognised that a completely different approach to that adopted for qualitative sampling [1] was necessary as shown in the Table1 and there were no existing guidelines within the ENFSI laboratories to cover quantitative sampling.

Analysis	Question	Answer	Sampling plan
Qualitative	Is the target substance present - at least in k% of individual items - to a required confidence level? [ENFSI Guideline]	Yes/No	Number of items to be tested individually
Quantitative	What is the average concentration of the target substance in the whole material?	X±U %	Primary sample: - size of increment - number of increments Analytical sample: - homogenisation process - weight of the sample

Table 1: Fundamental differences between sampling for qualitative and quantitative analysis

Impurity profiling involves the comparative analysis of minor components in illicit drugs and therefore needs an entirely separate sampling protocol, which is not covered in these guidelines.

The drugs investigated were:
- heroin
- cocaine
- amphetamine
- MDMA tablets
- cannabis (herbal Type I and Type II and resin)

During preliminary studies it became apparent that sampling for quantitative analysis is strongly influenced by two factors: the heterogeneity and the particle size of the original material. Therefore, before producing these guidelines it was necessary to characterise these factors for the most common illicit drugs [2, 3].

The basic goal in sampling for quantitative analysis is to maintain the average concentration of the drug in the material from its original seized state all the way through to the analytical sample.

In practice, where larger seizures of illicit drug preparations are concerned it is not possible to homogenise the whole material and modern analytical techniques only require a small amount (a few milligrams) of material. Therefore, it is necessary to carry out measurements on a smaller portion taken from the bulk, while maintaining the average drug concentration of the original seizure.

Sampling and analysis can be considered as two separate processes each with their own uncertainty. When a laboratory decides to demonstrate the overall performance of the whole process of quantitation, as part of the validation, it has to estimate the total uncertainty originating from both the analytical and the sampling process. The only exception, when sampling has no influence, is when the whole seizure is used as the analytical sample.

The probability of obtaining a *single* portion from the bulk material that perfectly represents the concentration of the parent material is extremely unlikely. If *several* portions (increments) are taken, their characteristics will vary due to the heterogeneity of real materials, but the average of the concentrations of individual portions will provide a better estimate of the concentration of the whole material.

It was found that the sampling problems caused by heterogeneity can be solved by using an incremental sampling protocol.

One of the main aims of our study was to bring the uncertainty of the sampling (expressed as the $RSD_{sampling}$) into line with the uncertainty of the analytical method applied (expressed as the $RSD_{analysis}$), which usually lies in the region of 2 to 5 % relative.

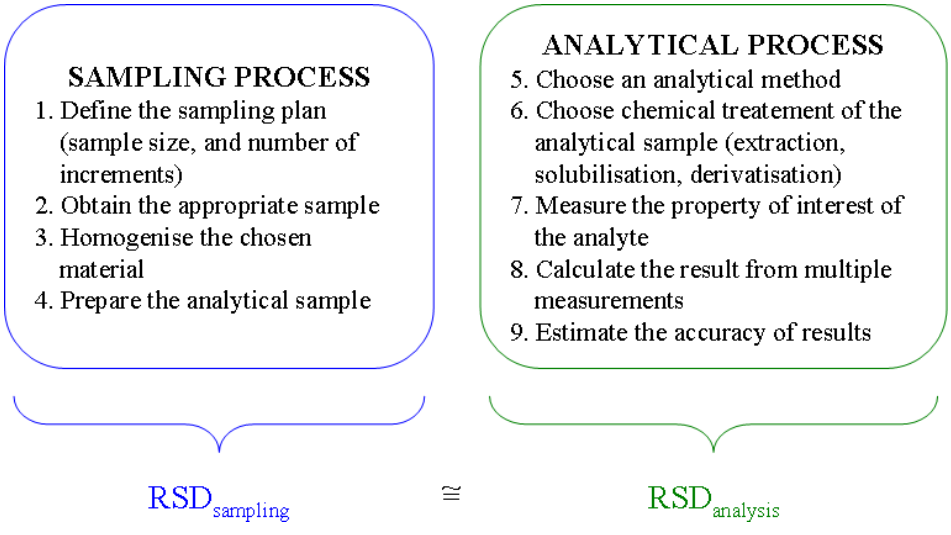


Figure 1: Outline of sampling and analytical processes.

Another important aim of our study was to provide a general method to calculate analytical sample sizes appropriate to the laboratories own analytical methods and required RSD's.

These guidelines provide sampling recommendations for most common illicit drugs. These represent minimum requirements, but there are tables and examples within the guidelines to help individual laboratories choose the number of increments needed to meet the specific requirements of their legal systems.

Note

Much more detail of the theoretical and experimental background to these guidelines is contained in three related articles [2, 3, 4] which the project team produced in 2012/13 and which have been published in 2013 and 2014. We recommend that these guidelines be read in conjunction with those three articles for a more detailed understanding of the quantitative sampling process.

3 Theoretical background

The most comprehensive theory on sampling for chemical analysis, which takes into account both the technical and statistical aspects of sampling was developed by Pierre Gy, along with others (Benedetti-Pichler, Pitard, Visman, Ingamells) [5-6].

This theory of sampling (TOS) was originally developed for the mining industry and provides a description of all the errors involved in sampling heterogeneous materials. It also describes how these errors can be evaluated and either minimised or eliminated.

3.1 Pierre Gy's Theory

We do not intend to expound on the detail of the whole theory, but merely to explain those parts of it that relate to our study.

Whether it is for convenience or economy, it is desirable that the mass of the gross (or primary) sample does not exceed that which is absolutely necessary.

The theory is based upon the mass of the primary sample and is essentially determined by

(1) The difference which can be allowed between the composition of the primary sample and the whole.

(2) The degree of heterogeneity of the material.

(3) The level of particulate size at which the heterogeneity shows itself.

A gas or liquid is heterogeneous only at the molecular level and it is the mass of the molecules themselves which determines the minimal mass of the primary sample.

The opposite is true for a particulate solid, such as an illicit drug powder or herbal material. In this case, each part of the material has a different composition. Heterogeneity takes place at a particulate level and the size of those particles can vary considerably. To obtain a really representative

primary sample, a certain number of particles have to be taken and this number depends on (1) and (2).

The 'largest' particles in these mixtures influence the size of the increment for sampling purposes. As a result of the way that powdered drugs are produced, these particles can be composed of one substance alone, when added separately in solid phase or mixtures originating from an extraction, precipitation or crystallisation process. The microscopic heterogeneity of a material depends on the size of the largest particles within that material. The presence (or absence) of a 'large' particle of pure active substance in a sample produces a higher (or lower) concentration than the average concentration of the material. The presence of a large particle of pure cutting agent will cause an equivalent deviation in concentration, but in the opposite way. Therefore, it is the size of the largest particle component and not the chemical composition of the particles which is relevant to obtaining a 'correct' sample [7].

Based on that theory to ensure that any sample taken from a seizure for quantitative analysis is truly representative of the whole seizure (i.e. that 'correct sampling' has taken place), several factors which influence the fundamental error need to be considered including [8-12]:

- constitution heterogeneity and particle size
- distribution heterogeneity
- mass reduction
- suitable comminution tools and equipment
- quality control

3.2 Heterogeneity

All real materials are heterogeneous. The question is: how and to what degree? There are two types of material heterogeneities which heavily influence the sampling of illicit drugs: distribution heterogeneity and constitution heterogeneity [13].

3.2.1 Distribution Heterogeneity

Distribution heterogeneity refers to the differences in how the pieces (fragments, particles or molecules) are distributed spatially i.e. how well

mixed or segregated the material is due to density, particle size, or other factors. Figure 2 demonstrates the distribution heterogeneity in a pile of dried cannabis.

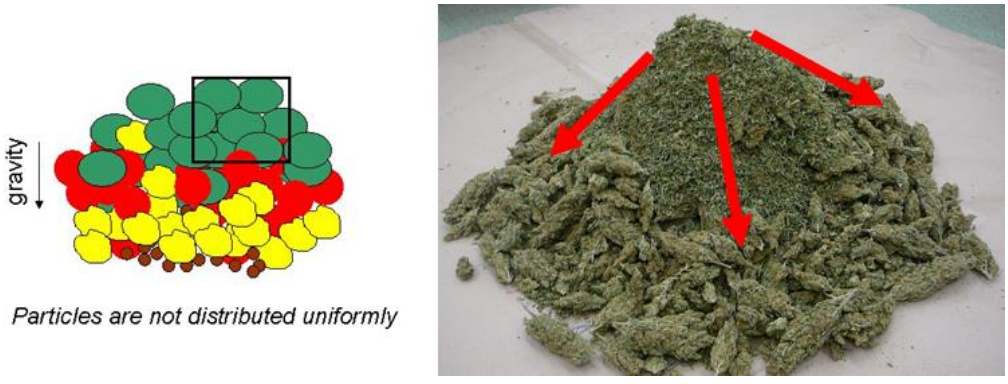


Figure 2: Illustration of the distribution heterogeneity of a sample of herbal cannabis.

Different pieces of material make different contributions to the average concentration (i.e. the concentration of the active ingredient is different in the buds, leaves and stems of cannabis). Furthermore, the distribution of different particles is not uniform in real materials.

These facts must be taken into consideration during the sampling process, to ensure that reliable results are obtained.

3.2.2 Constitution heterogeneity

Constitution heterogeneity refers to the differences in the constitution of the material i.e. how alike or different the individual particles or molecules are. It characterises the variation between individual fragments or particles (for solids) or between individual molecules (for liquids and gases). Figure 3 demonstrates the constitution heterogeneity for a real material.

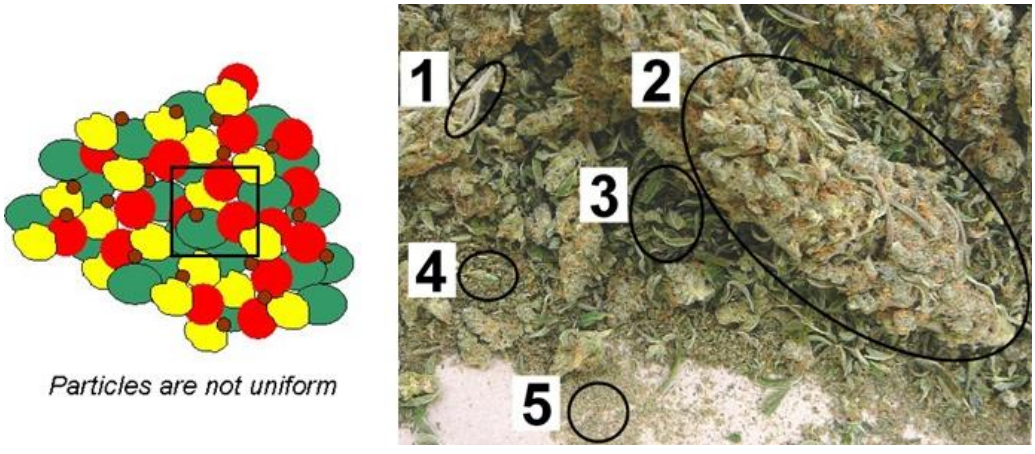


Figure 3: Illustration of the constitution heterogeneity of a sample of herbal cannabis: (1) = stems, (2) = buds, (3) = big parts of leaves, (4) = small parts of leaves and (5) = powder

3.2.3 Fundamental Error

Sampling is an error-generating process and heterogeneity is the **main** source of all sampling errors.

The fundamental error (FE) is the minimum error generated when we collect a sample of a given weight and is influenced by particle size.

The fundamental error is inherent in the compositional distribution of the sample material and cannot be changed without changing the nature of the sample [7]. This error can be reduced either by increasing the mass of the sample or by decreasing the size of the largest particles in it, by comminution.

Illicit drugs are encountered in many forms; they are predominantly solid particulate matter. Therefore the theory of sampling applied to divided solids (constituted of grains, which are in a solid state, such as sand, powders etc.) [5] is also appropriate for these materials.

The following equation (Equation 1) describes the relative variance of the fundamental error (FE):

$$s_{FE}^2 = IHL\left(\frac{1}{m_1} - \frac{1}{m_0}\right) \qquad \text{(Equation 1)}$$

where

m_0 is the lot mass (g) - represents the whole seizure

m_1 is the sample mass (g) - represents the primary sample taken from the seizure

IHL = constant factor of constitution heterogeneity

This equation can be also applied to the primary sample after comminution, and to the analytical sample.

Equation (1) can be simplified when the mass of the lot (m_0) is large compared to the mass of the sample weight (m_1 or m_n) and gives equation (2):

$$s_{FE}^2 = \frac{IHL}{m_1} \qquad \text{(Equation 2)}$$

Applying typical properties of particles in illicit drug preparations [5] the variation of fundamental error can be estimated as (Equation 3):

$$s_{FE}^2 = \frac{0.125cd^3}{m_1} \qquad \text{(Equation 3)}$$

where

- *c* is the mineralogic factor (or composition factor, related to a density in $g.cm^{-3}$),
- *d* is the nominal particle size or is the nominal size of fragments in the sample, equivalent to the maximum particle size in the lot to be sampled [14]. In practice, d is taken to be the screen size that retains 5% of the lot being sampled. For example, if a sample is sieved using a 2.5 mm aperture screen and 5% of the sample is retained on top of the sieve then *d* = 2.5 mm.

The diameter of the largest particles in the illicit drugs we studied was deduced by experiment.

The mineralogic factor *c* is defined as the maximum heterogeneity generated by the constituent (analyte) of interest in the lot. This maximum is reached when the constituent of interest forms two fractions, one containing the constituent of interest and the other containing none of that constituent.

c can be estimated by the following equation (Equation 4):

$$c = \rho_c \frac{(1-a_L)^2}{a_L} + \rho_{matrix}(1-a_L) \qquad \text{(Equation 4)}$$

The content of the analyte in the sample is defined as a_L a weight proportion [dimensionless], ρ_c is the density of particles constituting the analyte [g/cm^3], and ρ_{matrix} is the density of the matrix [g/cm^3] [15].

Name of the product	Density (g/cm^3)
Cocaine	1.25
Heroin	1.56 – 1.61
Amphetamine	0.95
MDMA	1.10
Methamphetamine	0.91
Delta-9-THC	1.13

Table 2: densities (density of the analyte ρ_c) of principal illicit drugs.

Name of the product	Density (g/cm^3)
Paracetamol	1.26
Caffeine	1.23
Lidocaine	1.00
Benzocaine	1.17
Procaine	1.23
Phenacetin	1.07
Hydroxyzine	1.18
Levamisole	1.31
Diltiazem	1.24
Mannitol	1.49
Inositol	1.28
Glucose	1.54
Lactose	1.53

Table 3: densities (density of the matrix ρ_{matrix}) of principal cutting agents found in illicit drugs.

The average density of the commonly encountered illicit drugs can be estimated as 1.0 g/cm^3. The average density of principal cutting agents can be estimated as 1.3 g/cm^3.

Relative variance of the fundamental error can be written as follows (Equation 5):

$$s_{FE}^2 = \frac{0.125\left(\frac{1-\left(0.7a_L + 0.3a_L^2\right)}{a_L}\right)d^3}{m_1} \qquad \text{(Equation 5)}$$

This last equation can be used to calculate the weight of the primary and analytical sample that need to be taken for quantitative analysis, with respect to the measurement uncertainty acceptable to individual laboratories.

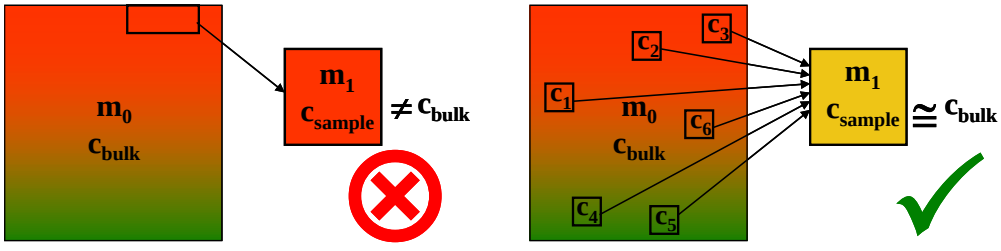
3.3 Reducing Sampling Errors and Obtaining a 'Correct' Sample

3.3.1 Reducing Sampling Errors

Sampling errors arise basically from differences in composition of particles of the material and from the segregation of different particles. Appropriate homogenisation of the whole material reduces the sampling error. For illicit drug preparations common techniques for homogenisation usually reduce the particle size of the material and reduce the segregation of different particles simultaneously [3]. In general the least sampling error is generated by homogenising the whole of the seized material and then taking a small analytical sample from that. This is the normal sampling practice for smaller amounts of illicit drug preparations.

However, where large seizures of illicit drugs are involved, laboratories have limited capabilities for the homogenisation of large amounts of material. The use of mortars and pestles of the size commonly used by analytical laboratories is not practical or reasonable for large quantities of materials (i.e. >100 g or kilograms). The volume and sample capacity of the containers of laboratory mixers and mills are also limited. Homogenisation of 5-10 kg or larger quantities of materials requires industrial-scale equipment. Laboratories usually take a portion from the bulk material as primary sample and this portion is homogenised using laboratory tools and equipment before acquiring the analytical sample.

Taking only one portion (increment) from an arbitrary position of the bulk material (i.e. from the top of the bag or pile) can generate error due to spatial heterogeneity of the material. Taking several increments from different spatial positions can reduce this error. Increasing the number of increments taken from the bulk (m_0) reduces the error arising from spatial (distribution) heterogeneity of the material. Taking increments from random positions is necessary to get a representative primary sample (m_1) and to guarantee an equal chance of each component getting into the sample (see Figure 4). In summary, this '*incremental sampling'* leads to a so called '*correct sample*' as explained in Pierre Gy's theory [5, 16, 17].



Arbitrary sampling Incremental sampling

Figure 4: Comparison of arbitrary and incremental approach

The size of increments taken from the bulk material has a significant influence on the sampling error: the smaller the size of the increments the greater the error arising from particulate structure and 'microscopic' heterogeneity of the material (see Figure 5). The optimum size of increment can be determined from the particle size data of the materials under investigation using sampling theory.

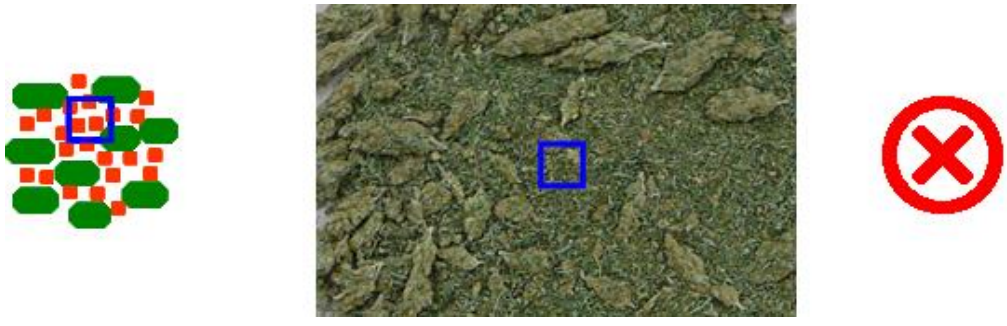


Figure 5: The adverse effect of inadequate increment size on sampling

Proper homogenisation of the primary sample provides suitable material for the preparation of an analytical sample. Particle size of the homogenised material is significantly smaller than in original material, consequently the relatively small amount of analytical sample can represent the same average concentration as the original material.

Errors arising during the sampling process cannot be corrected during the instrumental analysis, therefore using the principles of correct sampling is essential to obtain a reliable result for the seized material (Figure 6).

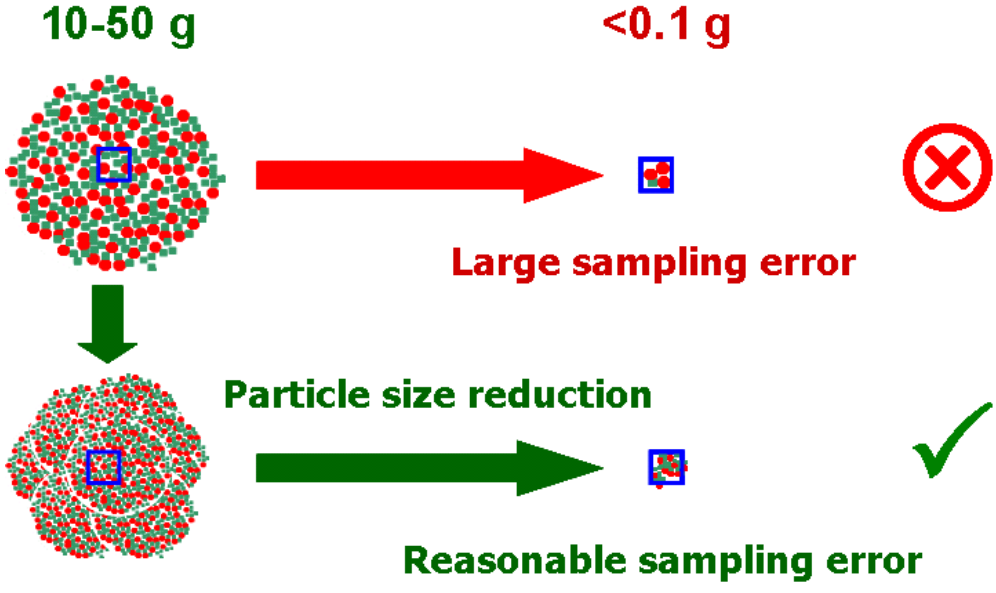


Figure 6: Effects of particle size on sampling error

3.3.2 Obtaining a 'correct sample'

When sampling, the aim is to obtain samples, throughout the whole mass-reduction process, that are representative of the bulk material. There are many definitions of a representative sample in the literature, ranging from broad qualitative to specific quantitative definitions. The key is to apply statistical inference, i.e. drawing conclusions about the population based on information from the sample. A sample can be considered representative of a population only to the extent that population characteristics can be inferred from the sample data. A sample by itself does not represent anything without the inference [7].

Pierre Gy's theory of sampling defined the notion of 'correct sampling'. The principle of 'correct sampling' for all samples (bulk solids, liquids and gases) is based on 2 statements:

(1) Every unit in the lot or population has an equal chance of being in the sample.

(2) The integrity of the sample is preserved during and after sampling (i.e. the sample stays the same between the time it is taken and the time it is analysed).

One of the requirements for 'correct sampling' is to carry out random sampling, because it gives a statistically unbiased result and a statistical estimate of the precision of the result. Samples taken using 'correct sampling' are representative by nature. Representative sampling is defined by the selection method and to produce reliable samples a correctly designed sampling protocol is required [5]. However, if it is practicable and applicable, the best approach for quantitative analysis would be to homogenise all of the bulk material.

3.4 Incremental sampling ('Spooning model')

The basis of our study was to apply the theory of incremental sampling to the practicalities of obtaining a 'correct sample'. Our practical approach we termed the 'spooning model' (Figure 7) and we aimed to answer the questions what size of spoon and how many spoons are necessary to obtain the 'correct sample'.

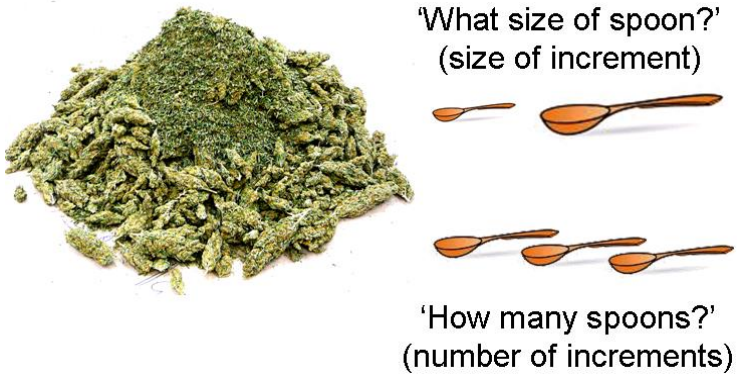


Figure 7: Illustration of incremental sampling ('spooning model')

The size of spoon is determined by the physical properties of the seized material. The number of spoons is determined by the heterogeneity of illicit drug material and the accepted level of uncertainty of the individual laboratory.

3.5 Mass reduction

It is in the nature of chemical analysis that the whole amount of material under investigation cannot be analysed. Modern analytical techniques only require a small amount (a few milligrams) of material. Therefore, it is necessary to carry out measurements on a smaller portion taken from the bulk. The sub-division of the bulk material to obtain a sample size suitable for analysis is known as 'mass reduction'. The 'mass reduction' process, from bulk to analytical sample, may be conveniently divided into several stages, as illustrated by Figure 8.

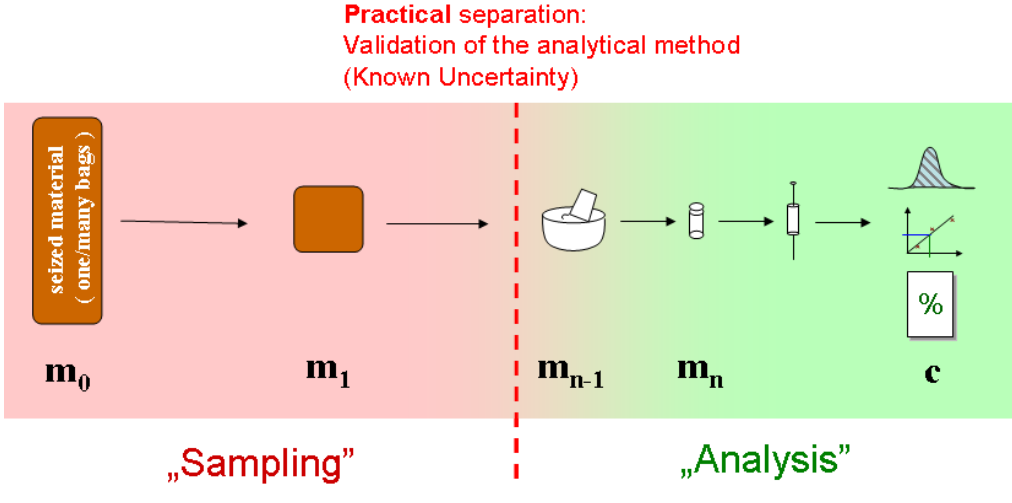


Figure 8: Mass reduction process from bulk material (m_0) to analytical sample (m_n).

The seized material (called bulk) has a given mass m_0 and its scale is generally from 1 g or less to 10–100 kg. The primary sample (m_1) is usually the

collection of one or more increments or units initially taken from the original material. Depending on the amount and type of seized material, several further mass reduction steps may be required to reach the analytical sample (m_n), which is the mass of material necessary for the measurement of concentration [12].

Once the analytical sample (m_n) is obtained any additional variance will usually covered by the validation process of the analytical method.

3.6 Sampling uncertainty, quality control of the sampling process and controlling the whole quantitative process

3.6.1 Sampling uncertainty

Sampling uncertainty is affected by two factors. Random effects which can be evaluated by statistical analysis [18] and systematic effects which can generate bias.

Random effects can be evaluated as the repeatability of the sampling process on the same material (sampling target) and uncertainty contributions arising from sampling can be estimated using mathematical statistical methods.

In practice sampling uncertainty is always combined with contributions coming from the uncertainty of the analytical method and the overall uncertainty of the whole process is the combination of the uncertainty arising from sampling and uncertainty of the analytical method (Equation 6):

$$u^2_{overall} = u^2_{sampling} + u^2_{analysis} \qquad \text{(Equation 6)}$$

Systematic effects cannot be easily evaluated as there are limited possibilities to compare different sampling protocols on the same sampling target and inter-organisational sampling trials are not available for illicit drugs. However, in practice by using sampling plans fit for ‘correct sampling’ systematic effects can be minimised.

Quality control of the sampling process is necessary to demonstrate the suitability of the sampling protocol applied. Statistical analysis of the results of duplicate or multiple sampling is a useful tool for the evaluation of sampling uncertainty and for continuous quality control [16, 18].

3.6.2 Controlling the whole quantitative process

The overall uncertainty of the whole quantitative process is the combination of the uncertainty arising from sampling and uncertainty of the analytical method (equation 6). When using these guidelines and when deciding whether you need a single or multiple primary samples, it is important to understand how the number of primary and analytical samples that you use will affect the whole quantitative process, as errors in certain parts of quantitative process will have a much greater influence on the overall uncertainty than others. The repeatability component of the measurement uncertainty can be estimated by the standard deviation of results from repeated measurements. Laboratories usually run duplicates to control their analytical process and the type of the duplication determines the type of cognisable effects. This is illustrated by the Figures 9, 10 and 11.

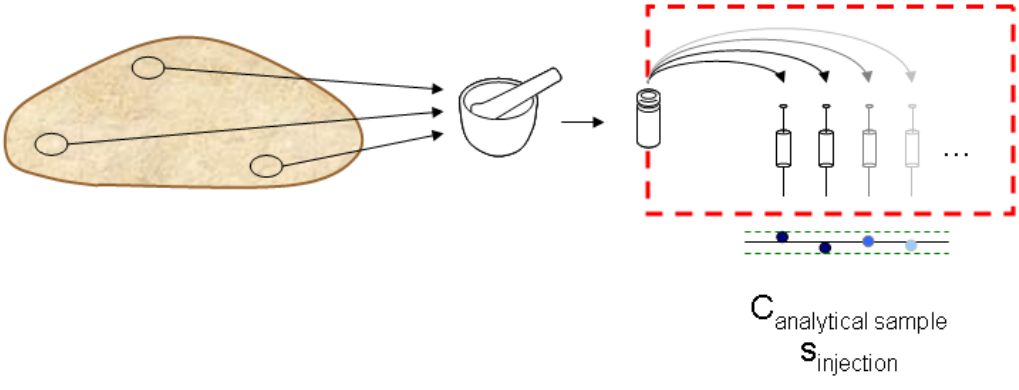


Figure 9: Multiple injection illustration.

Preparing a single analytical sample from a single homogenised primary sample and its multiple analysis gives information about repeatability of the analytical process, (usually the repeatability of the injection and integration), which only plays a small part in the uncertainty of analysis. With regard to the uncertainty of the sampling process, the seizure is assumed to have

typical or lower heterogeneity (as demonstrated by the heterogeneity study, see Table 4).

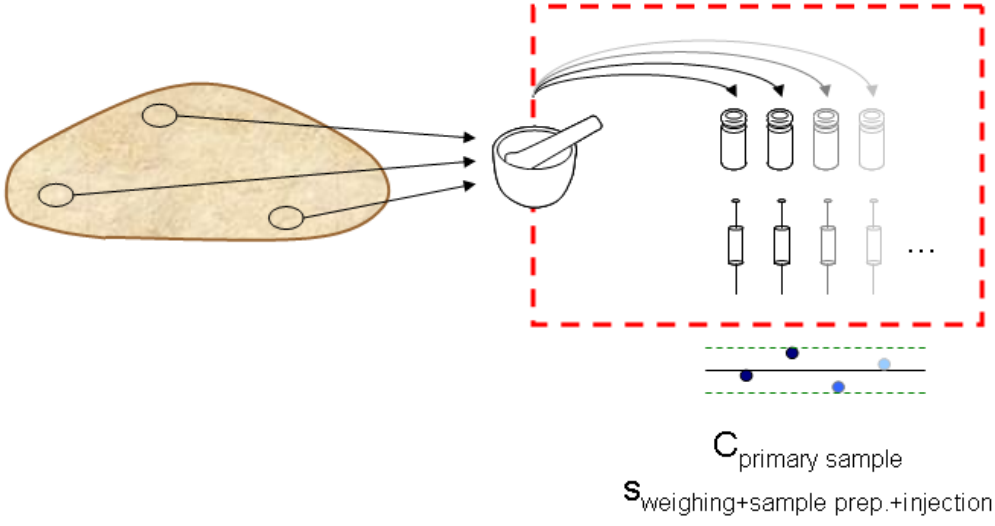


Figure 10: Multiple weighing illustration.

Preparing multiple analytical samples from one homogenised primary sample and their parallel analysis gives information about the repeatability of the corresponding analytical process (including sample preparation, injection, and integration) and reflects on the homogeneity of the primary sample. With regard to the uncertainty of the sampling process, the seizure is assumed to have typical or lower heterogeneity (as demonstrated by the heterogeneity study, see Table 4).

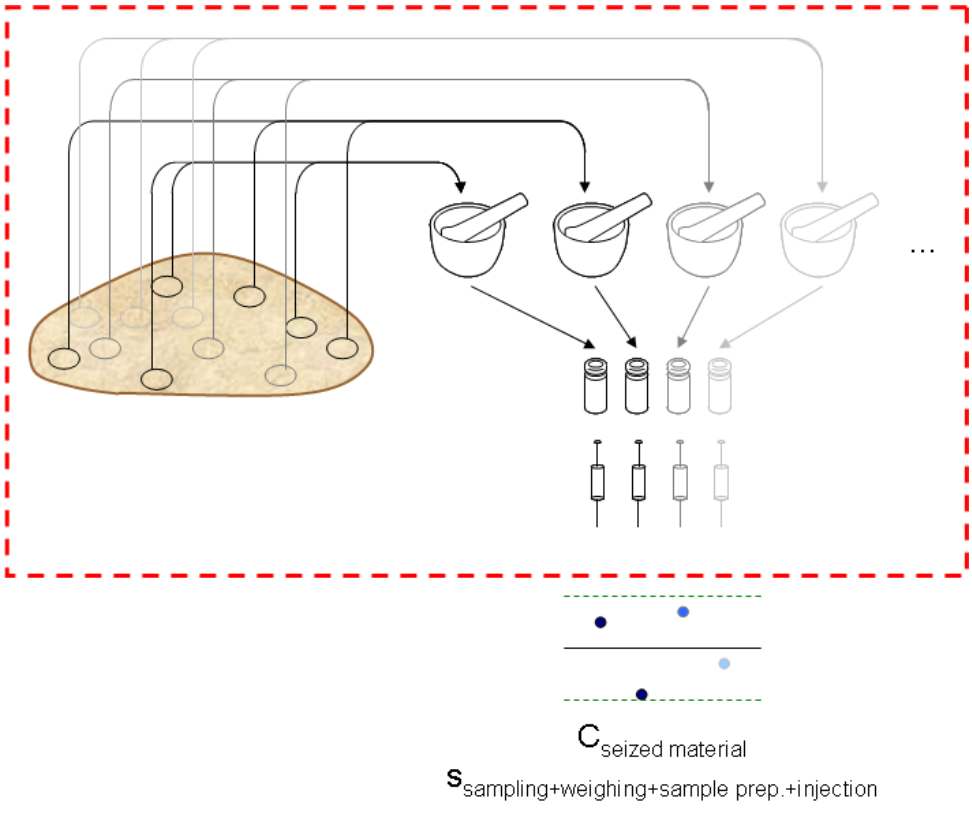


Figure 11: Multiple sampling illustration.

Preparing a number of single analytical samples from the corresponding homogenised primary samples and their analysis gives information about combined uncertainty of the applied sampling and analysis process. This method confirms that the sampling and analytical process were carried out on a repeatable way and that the seizure has typical heterogeneity (which is expected in most cases) and therefore no further sampling is required.

If the heterogeneity was high enough to affect the corresponding RSD (i.e. it has a value over the action limit of control chart), sampling would need to be modified (i.e. increase the number of increments needed for a primary sample).

4 Experimental results related to heterogeneity, particle size and mass reduction of illicit drugs

4.1 Survey on sampling and comminution techniques

A survey on the sampling and comminution techniques used by ENFSI member laboratories for illicit drugs was carried out in 2009.

This survey indicated that there is no consistent practice or approach in ENFSI laboratories for quantitative sampling. Incremental sampling was widely used, but arbitrary sampling, and coning and quartering were also common.

With regard to comminution, most laboratories used either mortar and pestle or laboratory mills for comminution of illicit drugs.

4.2 Heterogeneity study of illicit drugs in Europe

4.2.1 Background of the study

To set up a reliable quantitative sampling plan, information was needed on the heterogeneity of the most common illicit drugs.

Based on preliminary study we fixed the size of the portion (increment) at a 1 g level, for the purposes of our heterogeneity study. No significant effect from constitution heterogeneity would be expected from this approach. Therefore, this study could focus specifically on the distribution heterogeneity of drugs in their original form.

In order that we could obtain a broad overview we asked ENFSI laboratories to participate in a heterogeneity study of illicit drugs.

The heterogeneity of the untreated bulk drug material was studied for heroin, cocaine, amphetamine and cannabis herbal (Type I) and resin, using the following sampling procedures at a 1 g increment level.

For MDMA tablets, the sampling was performed analogously, using a 1 tablet level, rather than a 1 g increment level.

Herbal cannabis was also included at a 1 g level, which was thought to be equivalent to a 1 bud level.

In the routine analysis of seized powdered drugs, basically four different types of seizure are encountered; namely single blocks, multiple blocks, single

packages and multiple packages. We requested the ENFSI laboratories to sample all of these different types of real seizures (single block, multiple blocks, one big package and multiple packages).

The sampling method was prescribed by us and participating laboratories were asked not to deviate from the prescribed sampling process.

Three individual 1 g samples were taken randomly from seized material in its original state. Each of the three samples were homogenised and analysed individually in duplicate, resulting in 3 times 2 values, i.e. a dataset of 6 results. Samples taken from blocks were core samples (i.e. drilled through the whole depth of the block).

Samples were analysed using the routine quantitative method of the individual laboratories. Several laboratories submitted data on the heterogeneity of seized material from casework, which preceded this sampling study. Some previous results generated from sampling processes similar to those used in this study were included in our final experimental data.

4.2.2 Results

Heterogeneity data was collected from 21 European forensic laboratories between 2008 and 2009. Table 4 gives a summary of the dataset.

Drug	Laboratories	Seizures	Analytical results
Heroin	18	83	690
Cocaine	10	55	510
Amphetamine	9	37	226
Cannabis resin	7	24	150
MDMA tablets	5	16	99
Herbal cannabis	1	21	132

Table 4: Summarised dataset for the heterogeneity study.

Characterisation of materials by the variance or the standard deviation of the concentrations obtained in different parts of the material is consistent with the heterogeneity defined by the theory of sampling. Different seizures did not usually originate from the same batch or the same production procedure. Therefore, the heterogeneities of different illicit drug seizures varied considerably, compared to the variation of heterogeneity found in pharmaceutical products. This implies that a specific variance value for the heterogeneity of a particular illicit drug cannot be determined. However, a range of "typical" heterogeneities were determined and were essential for setting up an appropriate quantitative sampling plan.

Heterogeneity data from different seizures (different concentrations, matrices) can be compared as relative standard deviation (RSD) of experimental concentration values. For example RSD values for the concentration of 1 g increments in a multiple heroin block seizure fall in 0.5–30% range. Because of the wide range of RSD's and the number of data points collected, graphical exploratory data analysis tools were used to analyse the results.

A cumulative probability plot is a useful tool to compare distributions of heterogeneities for different drugs or different sorts of seizures (single and multiple blocks and bags). This plot can easily be created and has been applied in this study by sorting RSD values in ascending order; assigning the percentage of values that are less than or equal to a particular value and plotting the percentages (*y*-axis) against RSD values (*x*-axis).

For example: In case of 5 RSD values (1.4, 1.5, 1.8, 2.1 and 2.2), the percentage of RSD values less than or equal to the first value (1.4) is 20%, to the second value (1.5) is 40%, etc.

Cumulative probability plots of populations can be compared directly, regardless of the number of data points. These plots may also be informative for a low number of points and does not require any arbitrary setting (like category limits in histograms).

"Typical" heterogeneities were calculated as the square root of the average variance of experimental concentration's RSD values.

A so-called "maximum" heterogeneity has been determined from the cumulative probability plots (Figures 12 to 18). This is the heterogeneity that

corresponds to the 90% cumulative percentage (i.e. 90% of seizures in this study had a lower heterogeneity).

It should be noted that heterogeneity data also includes the repeatability of the analytical method which were typically 5% or lower for powdered drugs and MDMA tablets and cannabis resin and 10% or lower for herbal cannabis.

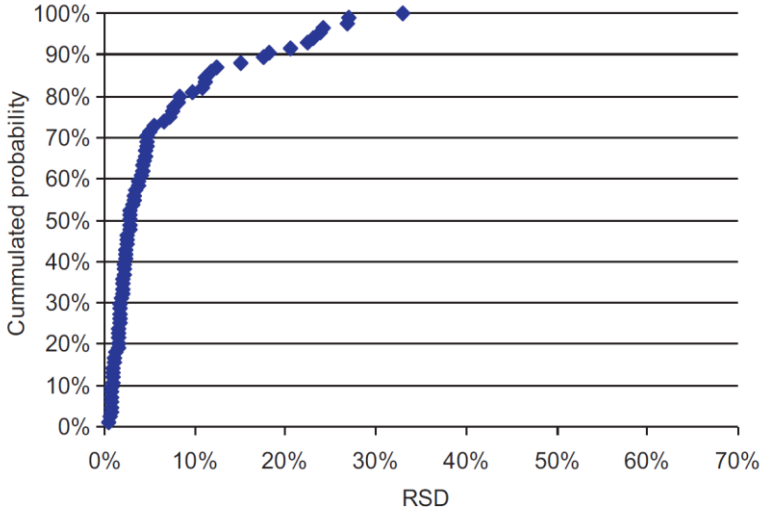


Figure 12: Distribution of empirical heterogeneity as RSD for heroin at a 1 g level

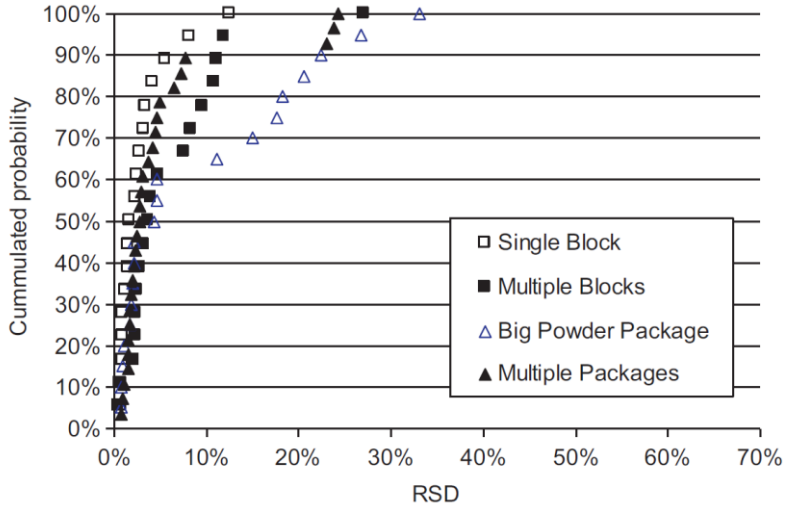


Figure 13: Distribution of empirical heterogeneity as RSD for heroin at a 1 g level, split into 4 different types of seizures.

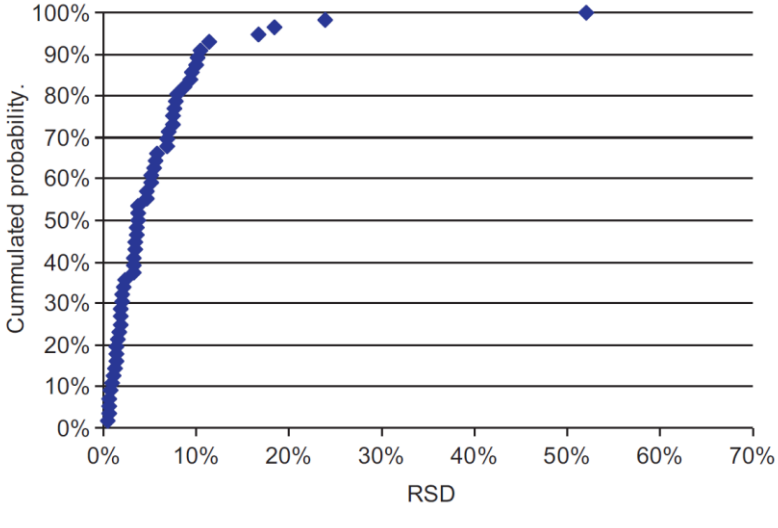


Figure 14: Distribution of empirical heterogeneity as RSD for cocaine at a 1 g level.

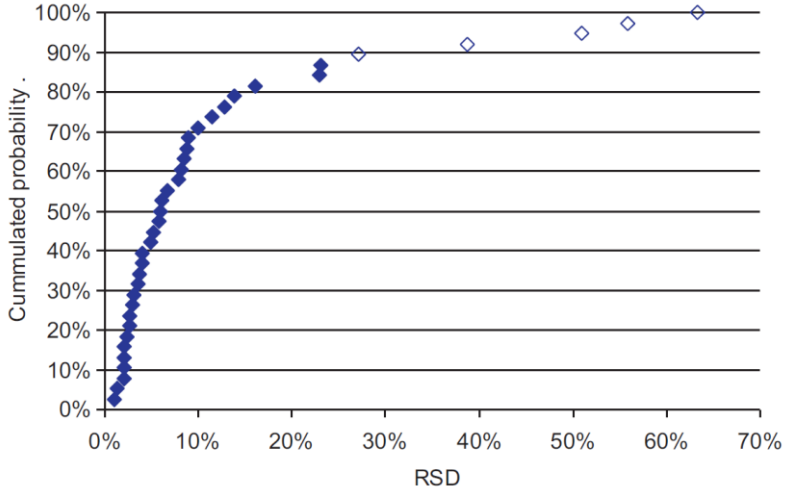


Figure 15: Distribution of empirical heterogeneity as RSD for amphetamine at a 1 g level.

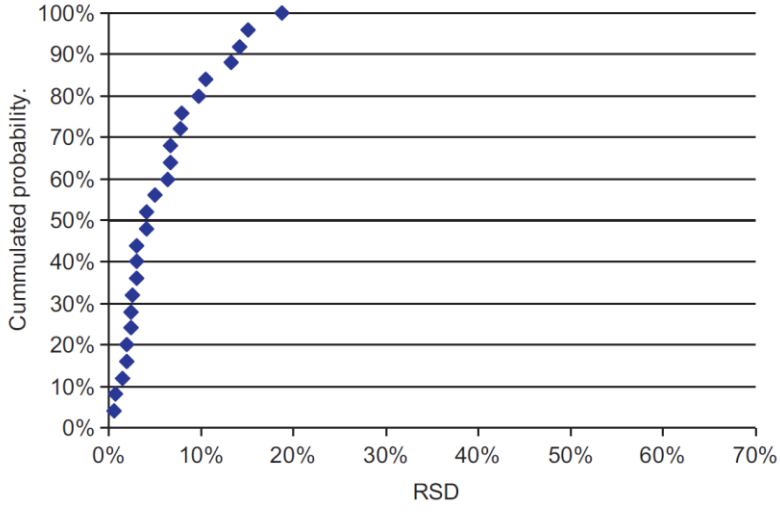


Figure 16: Distribution of empirical heterogeneity as RSD for cannabis resin at a 1 g level.

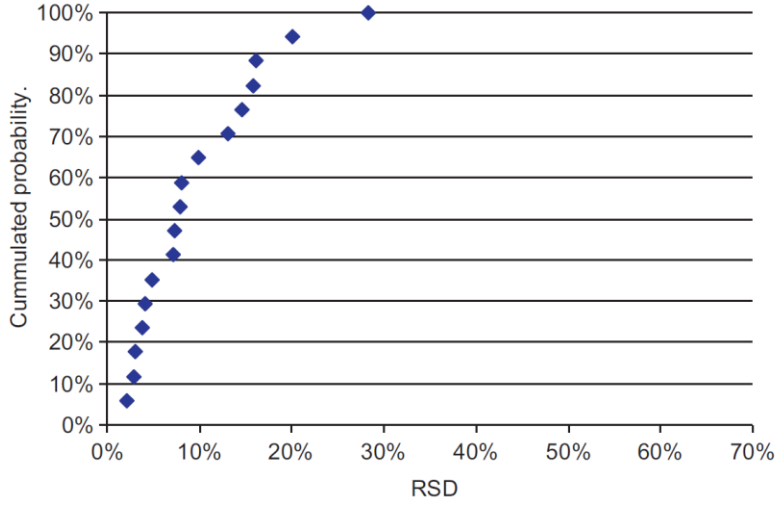


Figure 17: Distribution of empirical heterogeneity as RSD for MDMA tablets at a 1 tablet level.

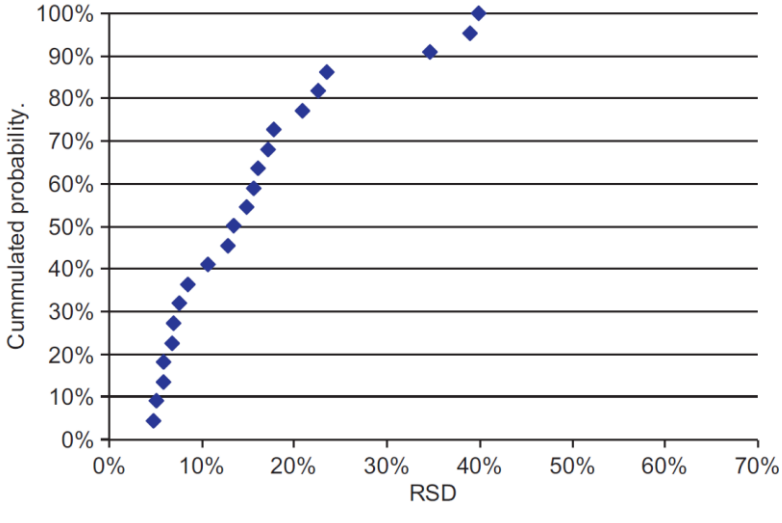


Figure 18: Distribution of empirical heterogeneity as RSD for herbal cannabis (buds) at a 1 g level.

Significant differences were identified between various types of heroin seizures (Figure 13). Heterogeneity in heroin blocks was lower than 15% RSD in most of the cases. The heterogeneity of the heroin blocks was approximately 30% in one case containing low concentration heroin powder (4%). Big packages of heroin were more heterogeneous than heroin blocks. Heroin powders are prepared for smuggling in block form. These blocks usually contain homogenous powder directly from the production. Big packages can be considered as diluted drugs because of lower concentration and higher heterogeneity.

5 of 38 amphetamine powders had low concentration (<5%) and uncommonly high heterogeneity (marked with unfilled markers in Figure 15). All of them were reported by two laboratories from the North-West part of Europe. These samples were considered as unusual materials and were ignored from the calculation of the “typical” heterogeneity for amphetamine.

Relative standard deviations characterise the “typical” and “maximum” heterogeneity values are presented in Table 5. “Typical” heterogeneities can be categorised in 3 groups: heterogeneity of powders and cannabis resin on a 1 g level is approximately 10%, heterogeneity of MDMA tablets on a 1 tablet level is 12%, and the heterogeneity of cannabis buds on a 1 g level is approximately 20%.

Drug	Typical heterogeneity [a] (as RSD %)	'Maximum' heterogeneity [b] (as RSD %)
Heroin	9.5	20
Cocaine	9.8	10
Amphetamine	8.9	25
Cannabis resin	7.8	15
MDMA tablets	12	20
Herbal cannabis (buds)	19	30

[a] Average variance of the experiments

[b] Approximately 90% of the results are below this level

Table 5: Summarised typical and 'maximum' heterogeneities for different drugs at a 1 g level.

4.2.3 Evaluation of the results

The "typical" and "maximum" heterogeneities represent the variances of randomly selected 1 g portions of "typical" and "very heterogeneous" materials. From an analytical point of view, when a laboratory takes 1 g portions from the material, the dispersion of the analytical results of each 1 g portion around the average concentration of the material can be characterised by these experimental heterogeneity values.

If a laboratory takes two 1 g portions (increments) from random positions in the material, combines them and analyses the combined sample, the result is equal to the average of the concentrations of the 1 g portions. The dispersion of the analytical results for combined samples can be characterised using the statistical formula for the calculation of the standard deviation of the mean (Equation 7):

$$s_{2\times 1g} = \frac{s_{1g}}{\sqrt{2}} \qquad \text{(Equation 7)}$$

If three increments are taken, the standard deviation of concentrations for the combined sample would be (Equation 8) :

$$s_{3\times 1g} = \frac{s_{1g}}{\sqrt{3}} \qquad \text{(Equation 8)}$$

Generalisation of these equations gives Equation 9:

$$s_{n\times 1g} = \frac{s_{1g}}{\sqrt{n}} \qquad \text{(Equation 9)}$$

Laboratories can use the experimental heterogeneity data from this study to set up sampling plan, according to their specific requirements (see Example 2).

Materials containing drugs in high concentrations are usually more homogenous than diluted drugs. Laboratories can chose to use different number of increments for concentrated samples (i.e. blocks of heroin) and diluted powders from the dealers or from the street level (i.e. big package and small packages of heroin and diluted amphetamine powders with uncommon heterogeneity).

If the materials in routine seizures are very heterogeneous the laboratory can decide to use the 'maximum' heterogeneity value.

For routine quantitative analysis of seized drugs the 'typical' and 'maximum' heterogeneity can be used easily by applying Equation 9 and consulting Table 6.

If the laboratory follows a protocol which fixes the number of increments, Table 6 shows what RSD values can be achieved, using that number of increments.

Number of increments (*n*)	s_{sample} (as RSD%) for material with typical $s_{heterogeneity}$ $s_{sample} = s_{heterogeneity} / \sqrt{n}$		
	20% heterogeneity ("cannabis buds" 1 g increment is too small)	**12% heterogeneity (tablets)**	**10% heterogeneity (powders and cannabis resin)**
5	8.9%	5.4%	4.5%
10	6.3%	3.8%	3.2%
15	5.2%	3.1%	2.6%
20	4.5%	2.7%	2.2%
50	2.8%	1.7%	1.4%
100	2.0%	1.2%	1.0%

Table 6: Formula and examples to reduce the expected RSD by increasing the number of increments.

The protocol followed by a particular laboratory may fix the number of increments or the maximum RSD allowed for an analysis. Equation 9 provides for both approaches.

The "1 g level" is problematic for herbal cannabis containing buds, leaves, stems and small fragments, due to the characteristics of that material, as shown in Figure 3 (particle size with different concentration, distribution and segregation). Therefore, a different approach for the correct sampling of herbal cannabis is needed.

4.3 Study on particle size of illicit drugs

4.3.1 Background of the study

This study investigated the effects of particle size during the mass reduction chain and the sample preparation process from the “primary sample” to the “analytical sample”, where the effect of particle size is most critical. It was based on the particle size measurement of different illicit drug materials in their original state and after homogenisation using manual or mechanical procedures.

Illicit powdered drugs and tablets generally take the form of mixtures. The psychoactive compound (drug) is usually diluted with other compounds namely adulterants e.g. caffeine, paracetamol, lidocaine etc. and diluents e.g. sugars, dimethylsulfone etc. It is the ‘largest’ particles in these mixtures, not necessarily the target drug, which will influence both the size of the increment in original form of the material and the size of the analytical sample after the comminution (particle size reduction).

The chemical identity of the largest particles in a drug material was not critical and therefore, only visual microscopic measurement was necessary to characterise these materials.

We investigated the size of the largest particles in the original, non-homogenised (uncomminuted) form of the drug preparation, and also the size of the largest particles after comminution. Amphetamine, compressed heroin and cocaine were included in this study. The size of the irregular shaped particles was determined from their lengths, i.e. their visually largest elongation was measured. Nominal particle size was estimated as the average size of the largest 50 particles.

For powdered drugs (heroin, cocaine and amphetamine) the particle size reduction was accomplished in two different laboratories, using either a mortar and pestle or a variety of mechanical devices (i.e. a ball mill, or different knife mills).

For herbal cannabis the particle size reduction was accomplished in a single laboratory by a variety of mechanical devices (i.e. knife mills). The particle size distribution of a cannabis seizure was characterised by a graded sieving process both in original and comminuted forms.

4.3.2 Results

Material	Treatment	Size range of the 50 selected particles [µm]	Average size of the 50 selected particles [µm]
Heroin Base (pressed block)	Original	391–909	607
Heroin Base (pressed block)	Original	410–993	604
Heroin Base (pressed block)	Original	442–959	634
Heroin Base (pressed block)	Original	457–953	660
Heroin Base (powdered)	Knife-mill (1)	153–605	296
	Mortar & pestle (6)	177–597	350
Heroin Base (powdered)	Knife-mill (1)	150–547	305
	Mortar & pestle (6)	108–488	283
Cocaine HCl (powdered)	Knife-mill (1)	99–467	224
	Mortar & pestle (6)	163–458	296
Cocaine HCl (powder)	Knife-mill (1)	123–507	267
	Mortar & pestle (6)	163–663	345
Cocaine HCl (powder)	Original	330–1092	588
	Knife mill (5)	119–576	260
	Ball-mill 1 min (3)	151–933	304
	Ball-mill 5 min (4)	113–445	239
	Mortar & pestle (6)	91–352	196
Amphetamine sulphate (powder)	Original	295–1537	590
	Knife mill (2)	110–779	361
	Ball-mill 1 min (3)	177–523	302
	Ball-mill 5 min (4)	132–498	235
	Mortar & pestle (6)	139–844	320

Table 7: Range and average size of 50 visually selected large particles in untreated and comminuted drug samples.

To show the visual effect of comminution, typical images from powder material (in original and comminuted state) are displayed in Figure 19.

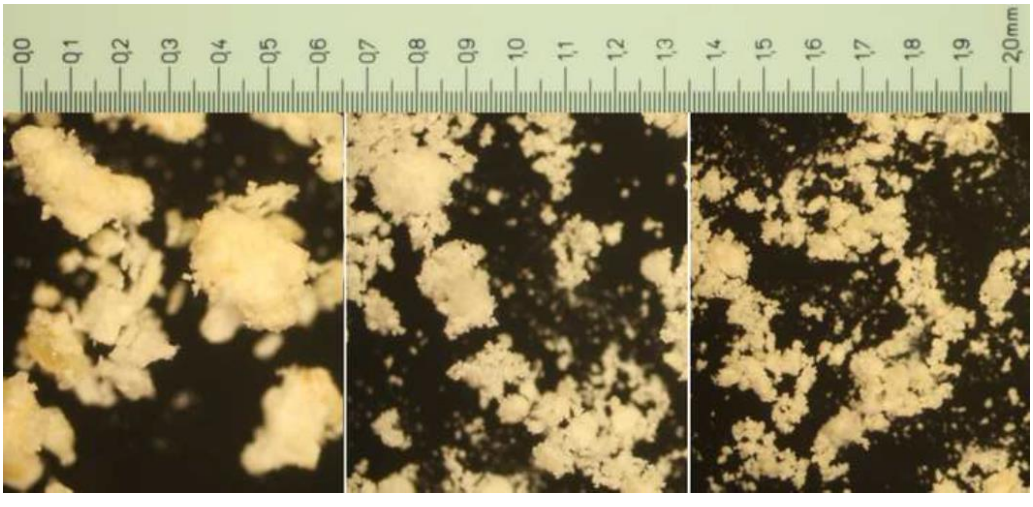


Figure 19: Images of particles from an amphetamine seizure in original (left) and comminuted states (middle and right).

As a general example of the determination of the nominal particle size, Figure 20 shows the diameters of 50 large particles from an amphetamine sample in its original (uncomminuted) state, in ascending order. In the same figure the effect of particle size reduction by the different techniques is shown. It can be observed there, as well as in Table 7, which includes the other investigated powdered drugs, that a decrease in nominal particle size of 50 - 60% can generally be achieved by comminution (i.e. from in the region of 600 µm to between 300 µm and 200 µm). However, it should be noted that the typical particle size after comminution would normally be much lower (i.e. in the region of 20–30 µm). It appeared that, in general, mortar and pestle (manual comminution) and knife mills (mechanical comminution) could be considered as comparably effective in that respect.

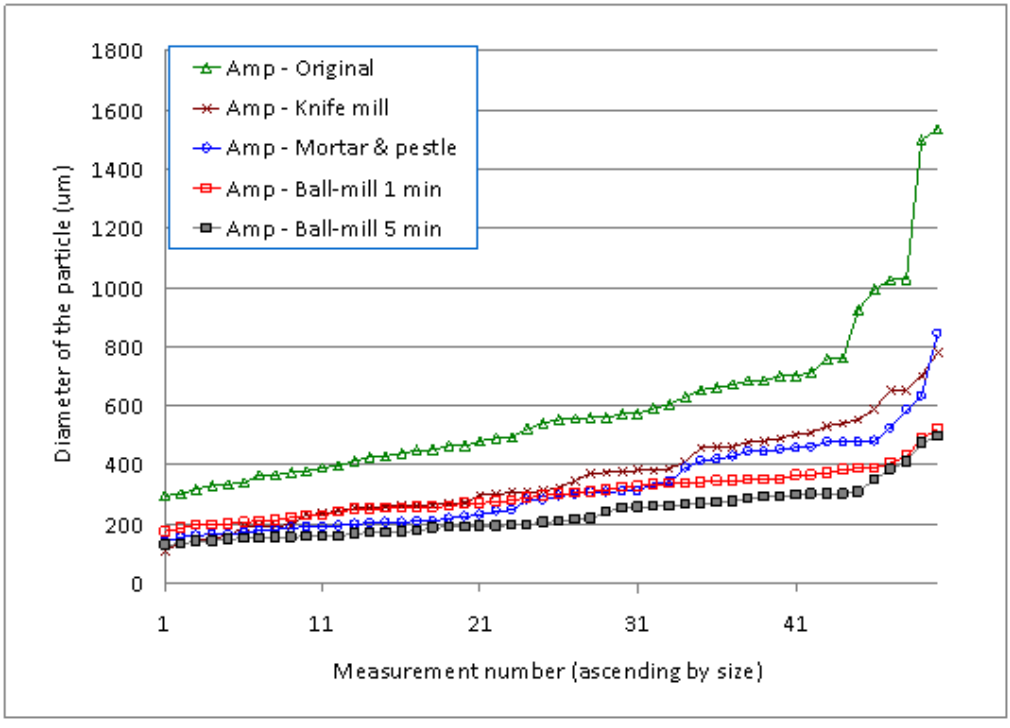


Figure 20: Particle sizes of illicit amphetamine preparation in original and comminuted states.

For herbal cannabis, the distribution of particle sizes is shown in Figure 21.

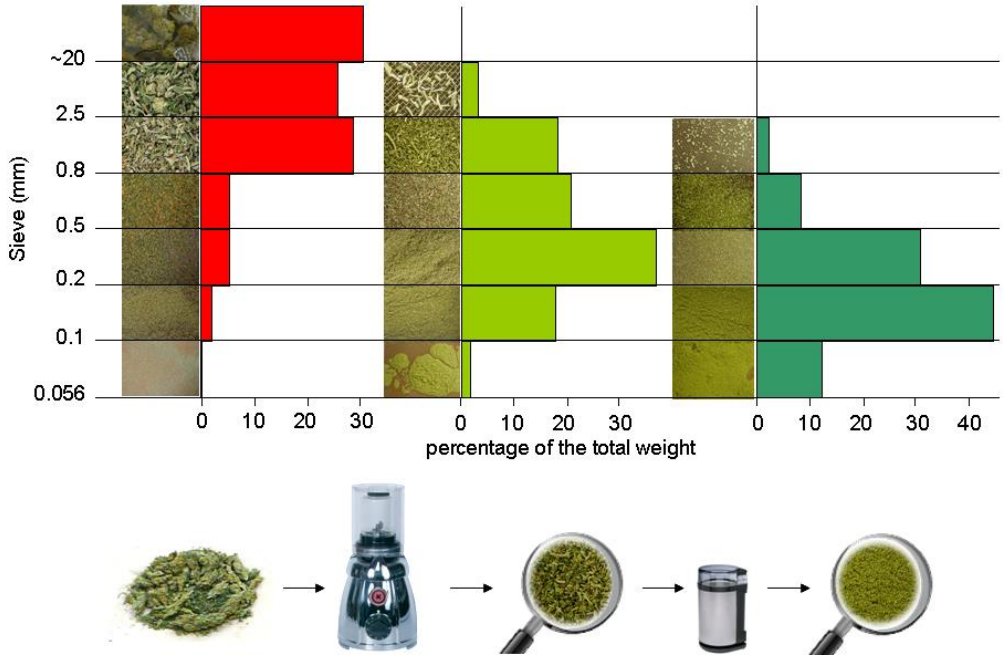


Figure 21: Image of particle size distribution of cannabis in original and comminuted state.

In its original form herbal cannabis has a very large spread of particle sizes, ranging from big buds several centimetres in diameter/length to much smaller fragments approximately 1 millimetre in diameter/length. Comminution by laboratory knife mill (No. 2 in Table 7) or domestic coffee blender (No. 5 in Table 7) reduces the particle size to below 500 µm, (i.e. by at least of a factor of 2).

The results for cocaine and heroin are shown below in Figures 22 and 23.

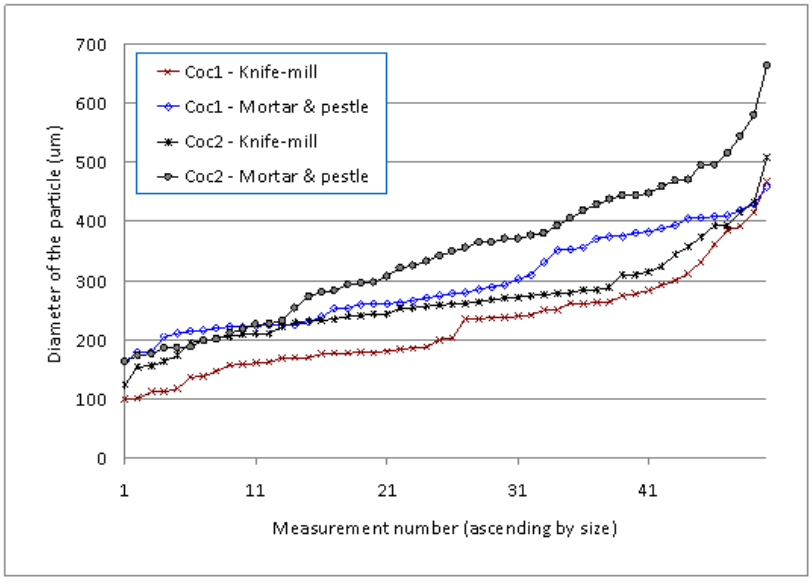


Figure 22: Particle sizes of illicit cocaine preparation in comminuted states.

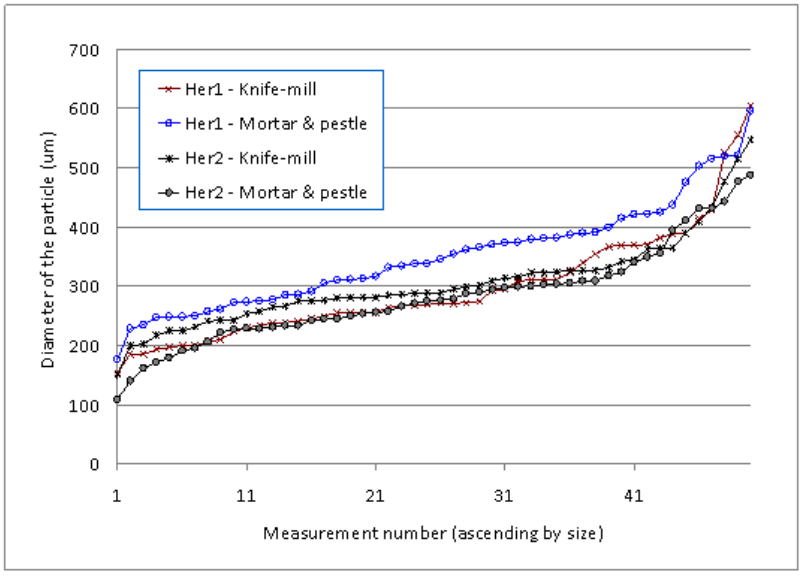


Figure 23: Particle sizes of illicit heroin preparation in comminuted states.

4.3.3 Evaluation of the results

One would normally expect the different methods of comminution (manual and mechanical) to be roughly equivalent. However, the results for cocaine and heroin shown in Figure 22 and 23 show that there may be advantages in terms of particle size reduction when using mechanical comminution.

By substituting the results of our particle size study into Equation 3, we confirm that the original assumption about 1 gram increment level, made before the heterogeneity study, was correct (see Example 2).

The examination of the illicit powdered drugs included in our study has shown that comminution, using various techniques reduces the nominal particle size to between 200 µm and 300 µm.

By applying these diameters and intended RSD values of between 2 and 5% to this mass reduction step we can evaluate, using Equation 3, the appropriate weight of analytical sample for quantitative analysis of the most common illicit drugs.

Based on the results of our study and after appropriate comminution we can generally state that:

An analytical sample weight of between 20–35 mg of an illicit powdered drug, with an assumed purity of 5%, would be considered appropriate and would generate an $RSD_{sampling}$ in the same region as the $RSD_{analysis}$ for a typical quantitative method for the analysis of the most common powdered illicit drugs. For herbal cannabis with an assumed purity of 1% THC, an analytical sample weight of approximately 200 mg would be appropriate.

5 Practical use of the sampling guidelines

5.1 Step by step guidance to sampling

At the beginning of these guidelines we made some general recommendations, which are applicable to the majority of illicit drug seizures (heroin, cocaine, amphetamine, MDMA tablets, cannabis resin and herbal cannabis Type I). However, should the laboratory requirements be more rigorous, then the following step by step approach to sampling and mass reduction should enable you to produce a correct analytical sample, suitable for quantitation for any of the named drugs.

- Decide if you can homogenise the whole sample or not
  - If you can, then carry out the appropriate homogenisation and produce your analytical sample(s) with reference to the particle size and estimated concentration of your target compound.
  - If you can't you need to do incremental sampling (on a 1 g level)

- To do incremental sampling you need to answer the following questions:
  - what target drug did you identify
  - what type of seizure is it (single block, multiple blocks, single package, multiple packages, tablets, cannabis)
  - what is your required $RSD_{sampling}$ with regard to your known $RSD_{analysis}$
  - is it necessary to perform double or multiple sampling (i.e. how many primary samples do you need)

- Go to 'Increment table' (Table 8), choose the RSD closest to the one you require and read off the corresponding number of increments required to obtain that RSD (see Example 1). Alternatively you can calculate the number of increments required for a specific RSD using the Equation 9 (see Example 2).

- Proceed to collect the required number of increments from your seizure (bulk material) for every primary sample that you need from it.
- Combine the required number of increments to produce your primary sample(s)
- Homogenise the primary sample(s)
- Produce your analytical sample(s) from the primary sample(s) with reference to the particle size and estimated concentration of your target compound.
- Perform your analysis(es)
- Check your results against your required $RSD_{sampling}$ using Equation 3 regarding the chosen weight of the analytical sample and the measured concentration of your drug.
- In case of multiple sampling, compare the results to your quality control limits (i.e. by control charts)

5.2 Recommended sampling plan for illicit drugs

Figure 24.A shows the sampling steps required for a single incremental sampling and Figure 24.B shows the steps needed where multiple incremental sampling is required. **It is up to individual laboratories to decide whether a single sampling is sufficient or whether they have more stringent requirements which need multiple sampling**. It is important to realize that when performing single sampling an assumption is made that the seizure has typical heterogeneity and the sampling process was carried out correctly.

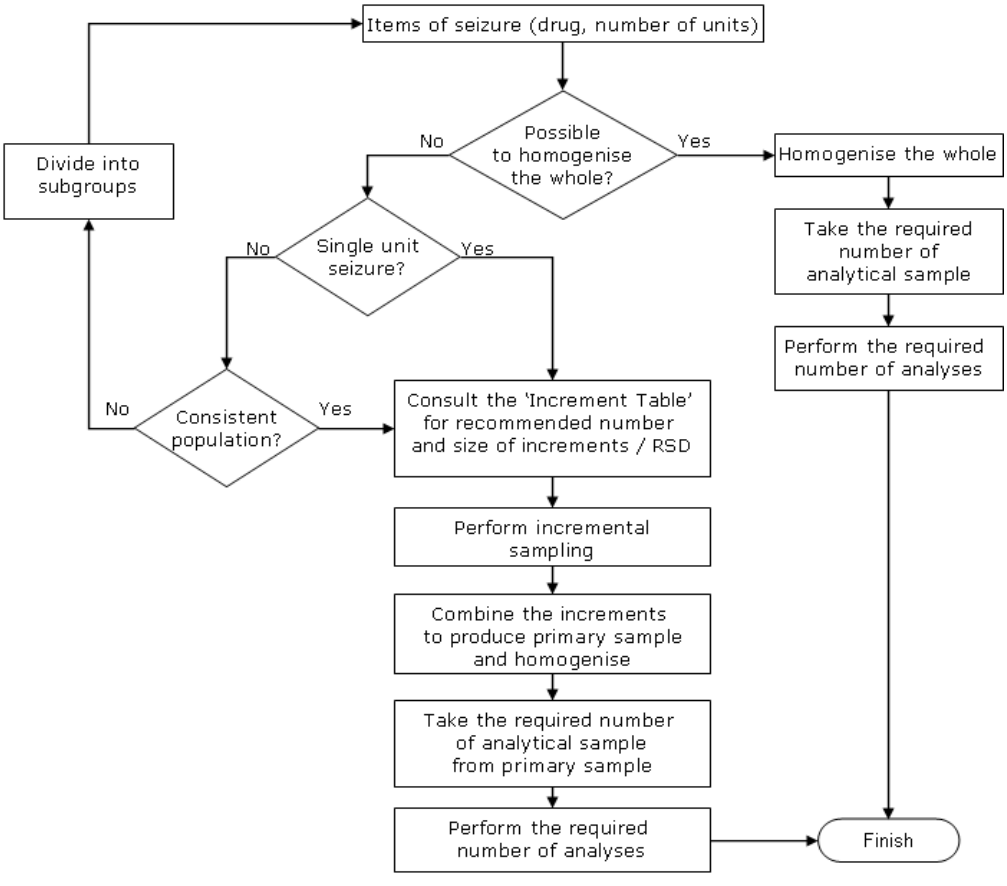


Figure 24.A: Flowchart for single sampling.

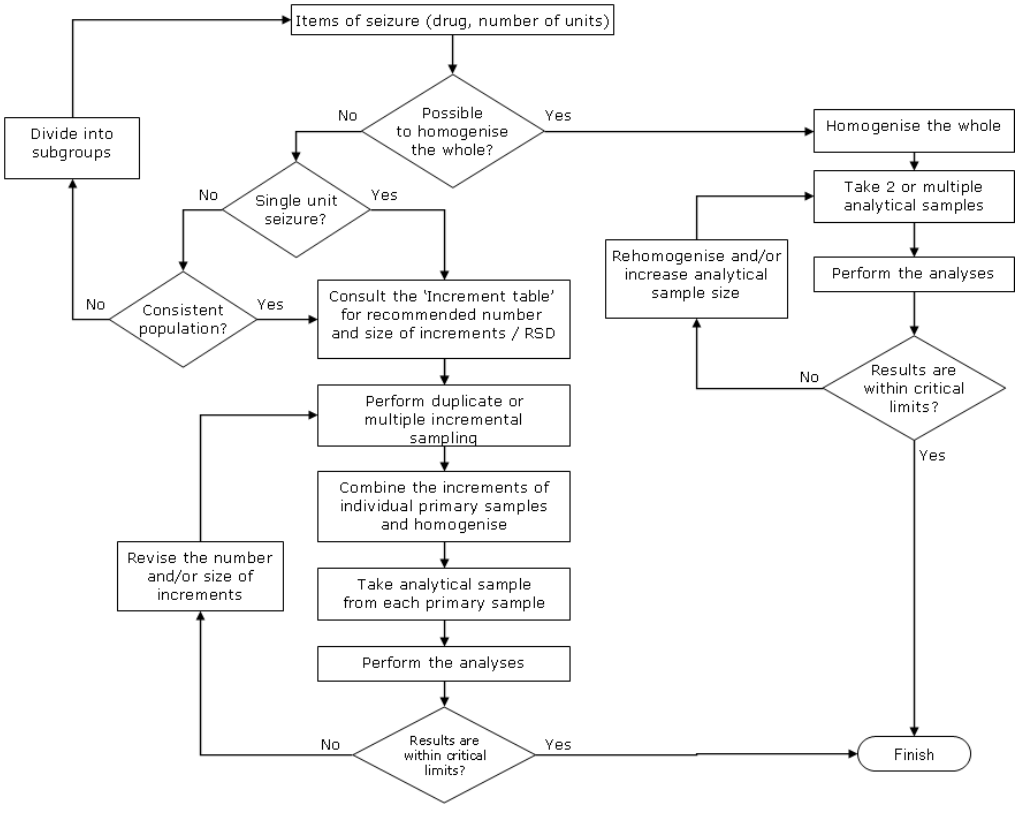


Figure 24.B: Flowchart for multiple sampling.

5.3 Determination of the number of increments according to required RSD

5.3.1 Using the 'Increment Table'

If the laboratory requires an upper limit of RSD Table 8 shows the number of increments required to achieve that RSD value.

If the laboratory follows a protocol which fixes the number of increments, Table 8 also shows what RSD values can be achieved, using that number of increments.

Number of increments (n)	s_{sample} (as RSD%) for material with typical $s_{heterogeneity}$ $s_{sample} = s_{heterogeneity} / \sqrt{n}$		
	20% heterogeneity ('Cannabis buds')	12% heterogeneity (Tablets)	10% heterogeneity (Powders & Cannabis resin)
5	8.9%	5.4%	4.5%
10	6.3%	3.8%	3.2%
15	5.2%	3.1%	2.6%
20	4.5%	2.7%	2.2%
50	2.8%	1.7%	1.4%
100	2.0%	1.2%	1.0%
Recommended size of increment	1 bud	1 tablet	1 gram

Table 8: 'Increment table': Formula and examples to reduce the expected RSD by increasing the number of increments.

Notes

Herbal cannabis with sticky resinous buds of similar size and appearance (referenced as Type I) can be considered as homogenous in large scale because of the similarity of particles (buds) and low risk of segregation of particles containing different amounts of THC.

Herbal cannabis seizures consisting of dry buds, leaves, stems and small fragments (referenced as Type II) can be very heterogeneous and subsequent handling of these materials may increases the heterogeneity. Therefore selection of the appropriate sampling procedure requires special consideration. The size of increments taken from the material must be larger than the biggest buds in the material to avoid delimitation and extraction error. A recognised alternative sampling procedure (e.g. coning and quartering) should be performed on this type of material, to guarantee the composition of the increment is representative of the bulk.

5.3.2 Using the equation for the standard deviation of the mean

If a laboratory takes several (n) 1g increments from random positions from a powdered drug or cannabis resin seizure, combines them to produce a primary sample and analyses a portion of the homogenised primary sample, the result will be equal to the average of the concentrations of the 1 gram portions. The dispersion of the analytical results for combined samples can be set up using the statistical formula for the calculation of the standard deviation of the mean:

$$s_{n\times 1g} = \frac{s_{1g}}{\sqrt{n}} \qquad \text{(Equation 9)}$$

The protocol followed by a particular laboratory may fix the number of increments or the maximum RSD allowed for an analysis. Equation 9 provides for both approaches.

For MDMA tablets the same equation can be used at the 1 tablet level and for cannabis buds (Type I) the same equation can be used at '1 bud' level instead of 1g level.

5.4 Selection of Increments

The selection of increments should be a random process to guarantee an equal chance of each part of the material getting into the sample. Incremental sampling is equally suitable for obtaining a primary sample from both a large single unit seizure and a multiple unit seizure containing the same material (see Figure 25).

Examples of incremental sampling of various types of drug seizure are given in the examples chapter of these guidelines.

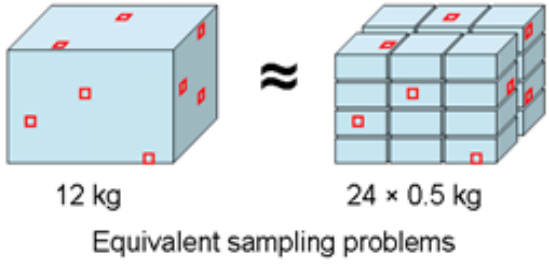


Figure 25: Incremental sampling of single and multiple unit seizures.

5.5 Sampling cannabis (Type II)

Herbal cannabis consisting of dry buds, leaves, stems and small fragments can be very heterogeneous due to the strong segregation of different particles. Small fragments can accumulate at the bottom of the container and can be placed on the top of the pile when the material is poured out of the container (see Figure 2). Segregation is accompanied by heterogeneity in the concentration of the THC, because of the different THC contents of various parts of the plant material (i.e. buds, leaves, stems). The critical particles for

sampling are usually the fragments in the size range of 1-2 mm, mainly leaf fragments of pistillate flowers containing higher level of THC than other fractions. Sampling methods which collect a reduced amount of these small particles underestimate the concentration of the whole material.

Application of a riffle splitter or a rotating divider is recommended to obtain a correct sample in many publications [9, 16, 17]. These pieces of equipment are available commercially, usually for smaller particle size. Common types of riffle splitters are available with slot sizes up to 5-10 cm, which are not appropriate for buds above 10-15 cm.

Reduction of the dimension of the sampling procedure is recommended to avoid practical problems [9]. Preparation of a long pile from the material in uniform width and height can be straightforward to access the desired part of the material. For heterogeneous herbal cannabis the creation of a uniform long pile and extraction of the slice in desired geometry were problematic in practice.

The comparison of various sampling processes for herbal cannabis Type II indicated that the best reproducibility was provided by the ‘cone and quarter’ method. [4] (A conical heap is formed from the material and the heap is flattened and divided into four identical quarters. Randomly selected opposite quarters are separated and mixed, the other two quarters are discarded. These steps are repeated to obtain the required amount of material (See Figure 26.) This method can be applied on up to 5-10 kilograms of herbal material.

Figure 26: Application of the 'cone and quarter' method.

5.6 Homogenisation of the primary sample and preparation of the analytical sample

The comminution methods generally applied by laboratories are pestle and mortar (manual) and various ball and knife mills (mechanical). Homogenisation of illicit drug materials (amphetamine, heroin, cocaine and herbal cannabis) using these techniques can reduce the nominal particle size down to between 200 and 300 μm.

The optimal size of analytical sample can be determined from the particle size of the homogenised material. An analytical sample weight of between 20-35 mg of an illicit powdered drug, with an assumed purity of 5% or higher, would be considered appropriate and would generate an $RSD_{sampling}$ in the same region as the $RSD_{analysis}$ for a typical quantitative method of analysis for the most common, powdered, illicit drugs. For herbal cannabis, with an assumed purity of 1% THC (tetrahydrocannabinol) or higher, an analytical sample weight of approximately 200 mg would be appropriate.

In seizures with low concentrations of target compound a larger amount of analytical sample or a smaller particle size are required to provide the same level of uncertainty (see Equation 5). In practice the further reduction of particle size would require more efficient comminution techniques.

Therefore, increasing the weight of the analytical sample would be more convenient.

5.7 Quality control of sampling

Errors arising during the sampling process cannot be corrected during the instrumental analysis. Therefore, using the principles of 'correct sampling' is essential to obtain a reliable result for the seized material. Quality control of the sampling process is necessary to demonstrate the suitability of the sampling protocol applied. The possibility of validating the sampling process of illicit drug preparations is limited, due to the lack of international, inter-organizational sampling trials and reference sampling targets. Primarily comparison with different sampling processes (i.e. homogenisation of the whole material) or detection of tendency in results of repeated sampling can be used for the evaluation of the bias of the sampling process. These techniques require numerous samplings and analyses and can be applied during the validation of the sampling process if necessary. Statistical analysis of the historical results from duplicate or multiple samplings is also a useful tool for the evaluation of sampling uncertainty.

Continuous quality control of the sampling process can be monitored by several methods. If the laboratory can homogenise the whole material, duplicate or multiple weighings are usually made to produce independent analytical samples. Relative difference or relative standard deviation of independent results can be compared to a control limit to verify the repeatability of the homogenisation and the sample preparation and the analysis process. Repeatability of the analysis (including the sample preparation) or long-term RSD of a control sample can be used for the calculation of the control limit.

The principal tool of the internal quality control of sampling is the replication. Comparison of the results from a repeated process can be applied both for the validation and for the continuous quality control of the sampling process. However, the bias of the sampling procedure cannot be detected by replication, but the bias can be minimized by using 'correct sampling' methods. Repeatability of the process can be controlled by the relative difference or relative standard deviation of results from duplicate or multiple samplings.

5.8 Control charts applied for quality control of sampling

5.8.1 Range control chart (R-chart)

Range control charts are constructed from the range or relative range of results originating from at least two complete replications of the sampling process on a sampling target (x_1 and x_2). Relative range can be used for samplings performed on different targets. Relative standard deviation of the sampling and analytical process (s_{rel}) can be estimated from the relative difference between two results of duplicate sampling ($d=|x_1 - x_2|/x_{avg}$ and $s_{rel}=d/1.128$, where $x_{avg}=(x_1+x_2)/2$). Duplicate results from at least 8 different samplings are recommended for the initial estimation of s_{rel}.

A one-sided control chart with warning limit $2.83s_{rel}$ and action limit $3.69s_{rel}$ can be used to control the routine sampling process (See 5.2 Principles for sampling validation and quality control in [8]).

5.8.2 Control chart for standard deviations (S-chart)

An S control chart can be constructed from the standard deviations of the results originating from replications of the sampling process on a sampling target. Relative standard deviation can be used for samplings performed on different targets. The relative standard deviation of the sampling process (s_{rel}) can be estimated as the average of the relative standard deviations of repeated samplings on different targets.

A control chart can be used to control the standard deviation of sampling process, using central line s_{rel} and warning and action limits, calculated according to the number of individual results per sampling target. For 3 individual results the warning limit is $s_{rel} + 1.045s_{rel}$ and the action limit is $s_{rel} + 1.568s_{rel}$ (see 6.3.2.1 Shewhart X-bar and R and S Control Charts in [10]).

5.9 Control the incremental sampling process

The replication of the incremental sampling process can be achieved with a small amount of extra work. For repeated samplings the increased number of increments can be taken in the same process, if the all the increments are

selected randomly. The increments combined into the required number of primary samples should be prepared for the analysis and analysed individually. The relative differences or relative standard deviations can be controlled by applying the appropriate control charts (R-chart for duplicate and S chart for multiple samplings). The relative differences or relative standard deviations can be compared directly to control limits calculated according to rules described above.

The alternative method for the quality control of incremental sampling process is the 'split absolute difference' (SAD) method, in which the normal number of increments to be combined as the primary sample is segregated at random into two equal sized 'splits', each of which is processed and analysed separately. The variance of the difference of two results is combined from the variance of the analysis and from the variance of sampling applied for the half of the number of increments [19].

The laboratory has the choice (or the duty, depending on its legislation) to demonstrate that the individual case sample to be quantified factually belongs to the majority of all case samples regarding its distribution heterogeneity by taking a **second combined sample** using the same sampling protocol. The two results (percentage of drug in the sample) must be in line within the chosen RSD of the laboratory or a value defined by legislation (see Figure 27).

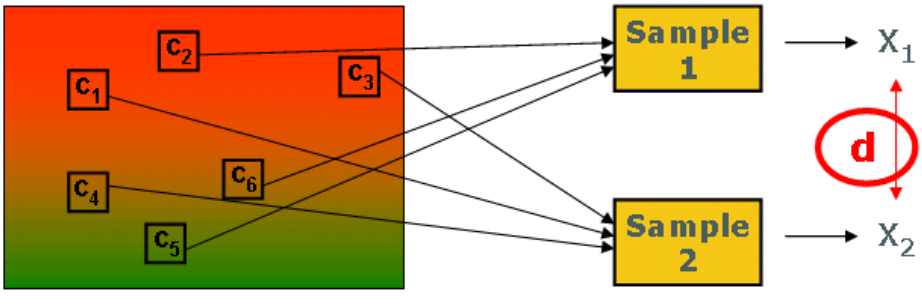


Figure 27: Comparison of results from duplicate incremental sampling.

Taking the example above, the overall measurement uncertainty $u_{analysis+sampling}$ (analytical part and sampling part, see equation 10) does not exceed 4.0%. If the difference between the two values is larger than a critical limit, the heterogeneity of the seized material is not a 'typical one' and a larger primary sample m_1 (larger increment size or number of increments) should be taken and homogenised.

$$u_{analysis+sampling} = \sqrt{(u_{analysis}^2 + u_{sanalysis}^2)} = \sqrt{(u_{analysis}^2 + u_{sampling}^2)} = \sqrt{(3^2+2.6^2)} = 4.0 \qquad \text{(Equation 10)}$$

Setting up a control chart shows that the case sample has a 'typical' heterogeneity and that the applied sampling procedure leads to a 'correct sample'.

The range (difference of two results) or standard deviation of multiple results can be evaluated using specific control charts [13]. A range chart (R-chart) can be applied to two results originating from duplicate sampling (see Figure 28) or an S-chart can be applied to results originating from multiple sampling (see Figure 29). Recording the relative differences of two results (X_1, X_2) on a range chart or recording the relative standard deviation of multiple results on an S-chart are applicable for control of the sampling process (control charts are specific to one particular drug, sampling process and analytical method).

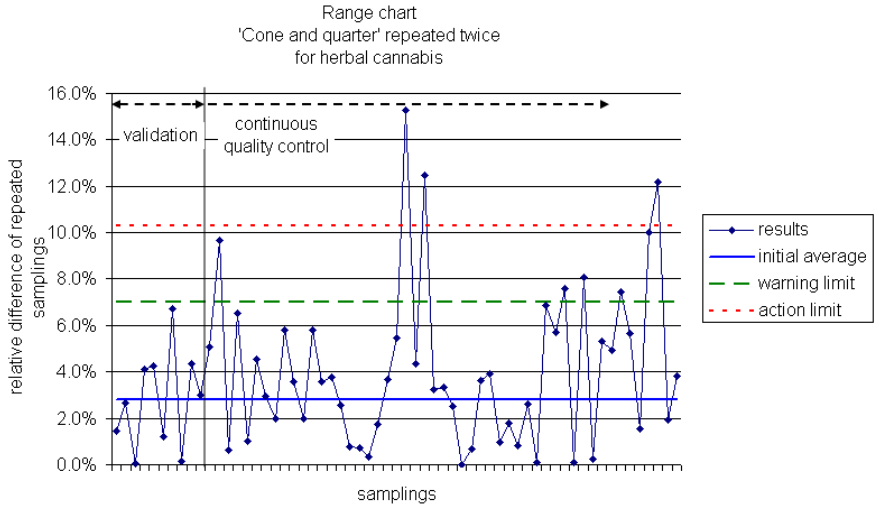


Figure 28: Control chart (Range).

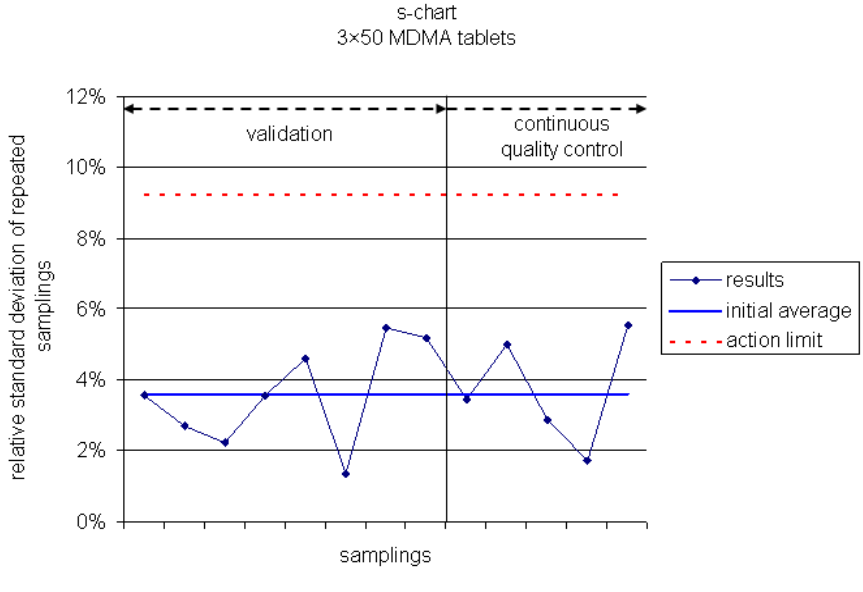


Figure 29: Control chart (Relative standard deviation).

If the relative difference or relative standard deviation of results from a specific seizure exceeds the action limit of the appropriate control chart then the sampling should be revised for that seizure.

During the validation of the sampling process control charts can also be used for the estimation of sampling uncertainty respecting the contribution of the repeatability component of the analytical uncertainty ($u_{analysis}$) [20].

5.10 Calculator for planning the sampling process

A calculator which allows you to easily calculate the number and size of increments for primary sample and the size of the analytical sample according to your individual requirements is available on the ENFSI website (www.enfsi.eu).

The calculator also contains preformatted control charts which can be used in the validation and quality control of the sampling process.

6 Examples

6.1 Example 1 - Using the 'Increment table' (Table 8) to determine the number of increments required for quantitative sampling

Assuming that

- a laboratory has a validated analytical method for quantitation of heroin with measurement uncertainty $u_{analysis}$ = 3 %
- if they want a measurement uncertainty for a whole method (sampling and analysis) to stay in the same range ($u_{analysis}$ ~ $u_{sampling}$)
- they need the sampling error ($u_{sampling}$) around <=3%.

Using the results shown in Table 8:

15 increments each of 1 g, selected randomly and combined together as primary sample (m_1) gives an estimated sampling error ($u_{sampling}$) of 2.6%, which is acceptable.

6.2 Example 2 - Calculating the number of increments using the Equation 9

If a laboratory wants a sampling protocol which results in an RSD of 5% or less, arising from sampling of "typical" blocks of heroin powder (heterogeneity 10 %), the minimum required number (*n*) of 1 g increments can be calculated using the following equation (based on Equation 9):

$$n = \left(\frac{s_{1g}}{5\%}\right)^2 = \left(\frac{10\%}{5\%}\right)^2 = 4 \rightarrow 4\,increments$$

Therefore 4 increments of 1 g should be taken, combined and homogenised to ensure the required RSD of 5% or less.

Applying the formula for the maximum heterogeneity (for heroin it is 20 %) will result in a sampling of 16 increments of 1 g.

$$n = \left(\frac{s_{1g}}{5\%}\right)^2 = \left(\frac{20\%}{5\%}\right)^2 = 16 \rightarrow 16 \; increments$$

Note

The "typical" and "maximum" experimental heterogeneity values are slightly overestimated, because they also include the standard deviation of the analytical process. Standard deviations arising from the reproducibility of the analytical process were significantly lower than the standard deviations of concentrations obtained from different portions of the original material. According to statistical rules, squares of standard deviations arising from reproducibility and heterogeneity should be combined. Therefore, the more heterogeneous the material, the smaller the contribution of the analytical standard deviation.

6.3 Example 3 - Comparison of the fundamental error with the RSD of analytical result

We can confirm that sampling illicit drugs for quantitative analysis at the 1 g increment level is correct by the following example:

If we consider:

- a concentration of amphetamine as a_L = 26.7% (w/w),
- a nominal particle size as d = 600 µm,
- an increment weight (sample weight) as m_1 = 1 g.

Using Equation 5 below applying values in appropriate units as a_L = 0.267, d = 0.06 cm and m_1 = 1 g:

$$s_{FE}^2 = \frac{0.125\left(\frac{1-\left(0.7a_L + 0.3a_L^2\right)}{a_L}\right)d^3}{m_1} \qquad \text{(Equation 5)}$$

we obtain s^2_{FE} = 0.00008 and a relative standard deviation (RSD) of the fundamental error FE s_{FE} = 0.0089 which is equivalent to 0.89%.

This RSD is much lower than one would expect for the measurement uncertainty normally associated with the validation of the analytical part of a quantitative method, which would normally be around 2 to 5%. Therefore, one could still obtain a normal RSD value of around 2 to 5% at amphetamine purities as low as approximately 1%.

Note

Taking multiple 1 g increments for the primary sample m_1 reduces the effect of constitutional heterogeneity and the fundamental error (FE) and therefore even lower RSD values should be attainable.

6.4 Example 4 - The effect of particle size on the weight of the analytical sample (for powdered drugs)

Consider an amphetamine sample (comminuted appropriately to give a nominal particle size of 300 µm) with a purity of 26.7% and a RSD value of 5%, using Equation 5, the appropriate analytical sample weight (m_n), will be 4 mg.

Even for amphetamine samples of much lower concentration (i.e. 5%) with an intended RSD of 5%, an analytical sample weight (m_n) of around 25 mg would be appropriate.

Note

Figure 30 below shows the relationship between the RSD and the analytical sample weight for a powdered drug of low purity (i.e. 5%) and a higher purity (26.7%) and a nominal particle size, after comminution, of 300 µm. Generally the higher the purity of the drug, the lower the acceptable analytical sample weight required.

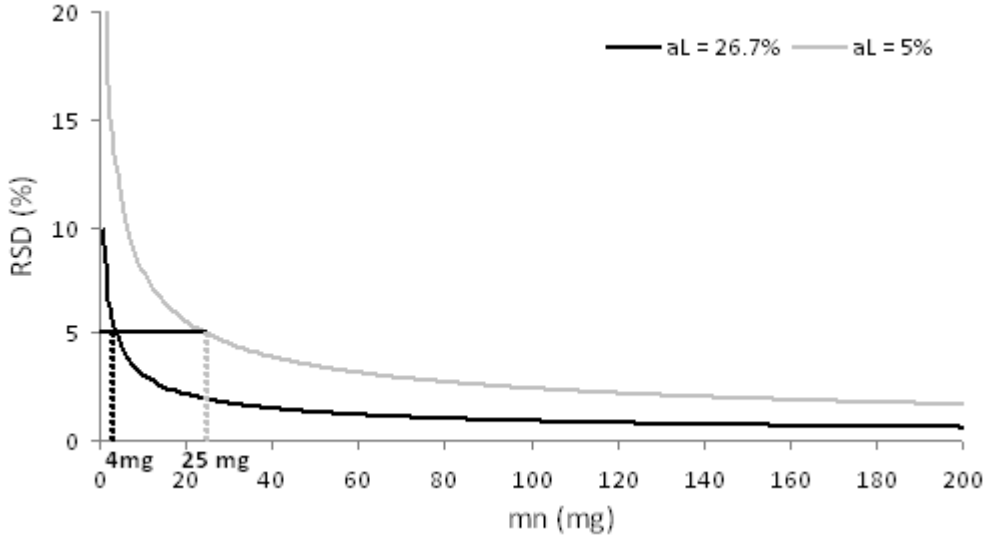


Figure 30: Relationship between RSD and sample weight (m_n) with 2 different concentrations (a_L = 26.7% and 5%).

During the analysis of materials where the drug concentration lies in normal concentration range the relative uncertainty of the analytical process should not vary significantly throughout that range. However for materials with very low concentrations of the drug (<1%) the relative uncertainty of both the analytical and the sampling process may be greater. For materials of this type the sampling plan which reflects the sampling uncertainty of the normal concentration range should be revised, if laboratory protocol requires it.

6.5 Example 5 - The effect of particle size on the weight of the analytical sample (for herbal cannabis)

Consider a herbal cannabis sample (comminuted appropriately to give a nominal particle size of 500 µm) with a purity of 1% THC and an intended RSD value of 5%, the appropriate analytical sample weight (m_n) will be 186 mg.

6.6 Example 6 - Sampling plan for single package of 25 g cocaine

Homogenise the whole material then take multiple weighings to produce analytical samples.

6.7 Example 7 - Sampling plan for single block of 0.5 kg heroin

Assuming that

- a laboratory has a validated analytical method for quantitation of heroin where the measurement uncertainty $u_{analysis}$ = 3 %
- if they want a measurement uncertainty for the whole method (sampling and analysis) to stay in the same range ($u_{analysis}$ ~ $u_{sampling}$)
- they need a sampling error ($u_{sampling}$) of <=3%.

Using the results shown in Table 1

- 15 increments each of 1 gram, selected randomly and combined together as primary sample (m_1) gives estimated sampling error ($u_{sampling}$) of 2.6%, which is acceptable.

6.8 Example 8 - Sampling plan for multiple blocks of heroin

The sampling plan will be similar to that in Example 7. Take 15 increments, each of 1 g, selected randomly from the blocks and combine together as primary sample.

Note

In case of more than 15 blocks, preliminary analytical information from the qualitative analysis of a specific seizure can be taken into account. Some techniques used for testing (or comparison) the composition of individual blocks can yield valuable information. For example, thin layer chromatography and the infrared spectroscopy are sensitive for several additional components (adulterants, cutting agents) and results from those tests can be compared visually to check the uniformity of the blocks. In case of uniform composition and average heterogeneity, 15 increments are adequate for the primary sample (see Figure 25). However, taking increments from all of the blocks increases the reliability of qualitative result.

6.9 Example 9 – Sampling plan for 100 small packages of cocaine each of 0.5 g

Option 1 - combine them all - if you can or if you are allowed to.

Option 2 - randomly select packages to get at least 15 g and combine together as a primary sample.

Option 3 - combine half the content of each package.

Note

Reducing the size of the increment increases the uncertainty contribution of one increment by the square root of the ratio of reduction (see Equation 2). Increasing the number of increments reduces the uncertainty by the square

root formula (see Equation 9). Taking the 15 g sample using an increased number of increments and a decreased size of increment yields approximately the same uncertainty as taking 15 × 1 g increments.

6.10 Example 10 – Sampling plan for 1000 MDMA tablets

Take a minimum of 20 randomly selected tablets, combine together to get primary sample.

6.11 Example 11 – Sampling plan for 5 kg of herbal cannabis (Type I)

Take a minimum of 50 randomly selected buds, combine together to get primary sample.

6.12 Comment about the sampling plan examples (6-11)

The examples shown above cannot cover every possible sampling situation laboratories may encounter in drugs casework. Where possible we recommend that you adhere to the 1 g increment for powdered drugs and cannabis resin, the 1 tablet increment for MDMA tablets and the 1 bud increment for herbal cannabis (Type I) combined to produce a primary sample of at least 15 g, 20 tablets, 50 buds. However, there may be instances when this is not possible. In these cases it is acceptable to take smaller increments and/or less of them to produce a primary sample. However, you need to be aware of the adverse effect that could have on sampling uncertainty. You need to use either the 'Increment Table' or the alternative Equation 9 for estimating the sampling uncertainty in those cases.

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Germany	Bundeskriminalamt
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Norway	National Criminal Investigation Service
Serbia	National Criminalistic National Centre, Ministry of Interior
Slovenia	National Forensic Laboratory
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NOTES

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