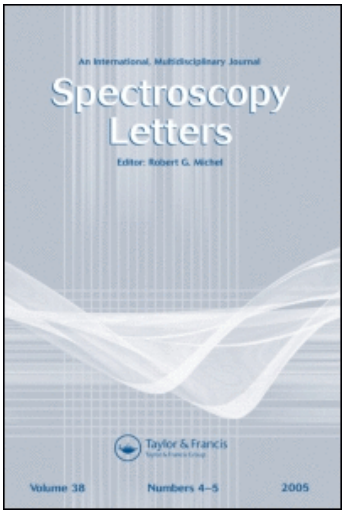


**Spectroscopy Letters**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

# Sampling and Sample Homogeneity as Introductory Topics in Analytical Chemistry Undergraduate Courses

G. L. Donati[a]; M. C. Santos[a]; A. P. Fernandes[a]; J. A. Nóbrega[a]
[a] Grupo de Análise Instrumental Aplicada (GAIA), Departamento de Química, Universidade Federal de São Carlos, São Carlos, SP, Brazil

**To cite this Article** Donati, G. L. , Santos, M. C. , Fernandes, A. P. and Nóbrega, J. A.(2008) 'Sampling and Sample Homogeneity as Introductory Topics in Analytical Chemistry Undergraduate Courses', Spectroscopy Letters, 41: 5, 251 — 257
**To link to this Article: DOI:** 10.1080/00387010802225138
**URL:** http://dx.doi.org/10.1080/00387010802225138

*Spectroscopy Letters*, 41:251–257, 2008

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010802225138

Taylor & Francis
Taylor & Francis Group

**Undergraduate Education Article**

# Sampling and Sample Homogeneity as Introductory Topics in Analytical Chemistry Undergraduate Courses

**G. L. Donati,**
**M. C. Santos,**
**A. P. Fernandes, and**
**J. A. Nóbrega**

Grupo de Análise Instrumental Aplicada (GAIA), Departamento de Química, Universidade Federal de São Carlos, São Carlos, SP, Brazil



**ABSTRACT** This work deals with the development of an experiment to evaluate the effect of sample characteristics on precision and accuracy of the sampling step. Parameters such as sample homogeneity, particle sizes, and sample mass were evaluated by analyzing the standard deviations (n = 3) obtained for Ca, Fe, Mg, Na, P, S, and Zn determinations in a breakfast cereal sample by inductively coupled plasma optical emission spectrometry. The proposed experiment can improve the assimilation of important concepts such as sampling, sample representativity, precision and accuracy by undergraduate students in analytical chemistry laboratory courses.

**KEYWORDS** accuracy, chemical education, grinding, ICP OES, precision, sampling

## INTRODUCTION

The past decades have seen an astonishing development of instrumental analysis, and the determination of trace elements is gradually becoming feasible even in relatively small laboratories.[1]

The main steps of an analytical procedure are sampling, sample preparation, and analyte determination. The errors associated with this last step have become relatively small, especially due to improvements in instrumental techniques.[2] Sample preparation represents the second largest source of error (100–300%).[3] However, due to the gradual dissemination of microwave-assisted procedures[4] and more attention being paid to this step in the past few years, it is possible to have a better control of the errors associated with this analytical step.[5] On the other hand, the sampling step still requires more attention by analytical chemists working in either educational or industrial areas.

Sampling has become the most critical step affecting the quality of the results. It still remains as a challenge to analytical chemists, even considering the wide acceptance of the idea that the accuracy of an analysis cannot be better than the accuracy of the sampling step. Problems in this particular step can cause errors as great as 1000%,[3] affecting both precision and accuracy of the whole analytical procedure.[6]

Received 30 July 2007;
accepted 26 March 2008.

Address correspondence to J. A. Nobrega, Departamento de Química, Universidade Federal de São Carlos, Caixa Postal 676, 13560-970, São Carlos, SP, Brazil. E-mail: georgedonati@yahoo.com.br

Taking into account all aspects related to the sampling step, it is extremely important to discuss topics such as sample homogeneity, particle sizes, and sample mass in freshmen classes of analytical chemistry. It is essential to demonstrate the effects of such parameters on sample representativity and, ultimately, on precision and accuracy. However, undergraduate students frequently have great difficulty to understand concepts related to representativity. Instructors, on the other hand, frequently have problems to demonstrate practical aspects such as how poorly representative is a sample aliquot of such small mass or how unreliable are the results for an analysis in which no appropriate sample homogenization was employed.

Several authors have developed experiments emphasizing the importance of representative samples for method development. Vitt and Engstrom[7] proposed an experiment to introduce basic concepts of sampling and statistical analysis to chemistry students by using a mixture of two different colored glass beads. The beads were placed in three beakers of different sizes (10, 20, and 50 mL). After the investigation of the amounts of beads and colors in each one of the beakers, the students noticed that the standard deviation for a specific color decreased when the size of the beaker increased. Similar results were obtained by Ross[8] using M & M candies. The author also investigated the effect of particle sizes on sample representativity. In this experiment, students noticed that sample segregation can occur during the sampling step, (i.e., big candies were generally present in larger amounts, with smaller deviation patterns than the small ones in the sampled mass). Hartman et al.[9] also investigated the effect of the sample homogeneity on precision and induced the students to use statistics to determine the number of replicates needed to obtain results in a specified confidence limit.

Most works about sampling have been done by using macroscopic models, (i.e., candies, glass beads, etc.) to show the importance of sample homogeneity, particle sizes, and representativity. Although this approach is useful to describe aspects related to the sampling step, students have difficulty to relate these models with real samples. Frequently, they do not recognize the relevance of such parameters in real-life chemical analysis, and few works have dealt with this issue.[10,11]

The goal of the work here described is to investigate the effects of sample homogeneity, particle sizes, and sample mass on precision obtained in element analysis of a breakfast cereal sample. Three methods of grinding for particle sizes reduction and homogenization were evaluated: cryogenic, knives, and mortar and pestle. The objective is to use real samples to introduce important topics such as sampling and sample homogeneity in undergraduate analytical chemistry laboratory courses.

## MATERIALS AND METHODS

### Reagents and Samples

All solutions were prepared by using analytical grade reagents and deionized water (Milli-Q water, 18 MΩ cm; Millipore, Bedford, MA, USA). All glassware and polypropylene flasks were previously washed with neutral soap, soaked in a 10% v/v nitric acid (Merck, Darmstadt, Germany) solution, and rinsed with deionized water. Reference solutions were prepared by diluting stock solutions containing 1000 $\mu g\,mL^{-1}$ of each element (Tec-Lab, Hexis, São Paulo, SP, Brazil) with 1% v/v $HNO_3$.

A breakfast cereal sample was employed to evaluate the effect of sample mass and grinding process on homogeneity. This sample is composed of 12 constituents: corn flakes, toasted oat, sugar, oat flakes, raisins, dehydrated apple, rice flakes, powdered malt, honey, salt, wheat bran, and grated coconut. These constituents differ in their amounts, chemical composition, particle sizes, hardness, and grindability.

### Instrumentation

The analysis was performed by a VISTA AX simultaneous inductively coupled plasma optical emission (ICP OES) spectrometer with axial view configuration (Varian, Mulgrave, Australia). The sample introduction system consists of a V-groove nebulizer and a Sturman–Masters type spray chamber made of polytetrafluorethylene (PTFE). The operating parameters and the selected analytical lines are listed in Table 1.

A microwave oven (model Ethos 1600; Milestone, Sorisole, Italy), equipped with 120 mL Teflon-PFA vessels was used for sample acid digestion.

**TABLE 1** Instrumental Parameters for ICP OES with Axial Viewing

| Operating parameters | |
|---|---|
| RF generator (MHz) | 40 |
| Power (kW) | 1.2 |
| Plasma flow rate (L $min^{-1}$) | 15 |
| Auxiliary flow rate (L $min^{-1}$) | 1.5 |
| Nebulizer flow rate (L $min^{-1}$) | 0.90 |
| Sample flow rate (mL $min^{-1}$) | 1.0 |
| Injector tube diameter (mm) | 2.4 |
| Spray chamber | Sturman–Masters |
| Nebulizer | V-groove |
| Emission lines (nm) | Ca II 396.486 |
| | Fe II 238.204 |
| | K I 766.498 |
| | Mg II 280.265 |
| | Na I 588.995 |
| | P I 177.434 |
| | S I 180.669 |
| | Zn I 213.857 |

A knives mill (model MR340, Microtec, Ribeirão Preto, SP, Brazil), a cryogenic mill (model 6750, Spex Certiprep, Metuchen, NJ, USA), and an agate pestle and mortar were used for sample grinding.

## Laboratory Activities

Three different grinding processes (i.e., knives, cryogenic, and mortar and pestle) were evaluated for sample homogenization. The main differences between these procedures are listed below:

*Knives mill*: It breaks up bulky, soft to moderately hard, fibrous plant specimens and cellulose-containing samples. Cutting and shear forces lead to comminution. The material only remains in the grinding chamber until the necessary degree of fineness is reached. The ultimate degree of fineness achieved depends on the exchangeable screens present at the sample output.[5] In this procedure, only the mass necessary for study was ground without any temperature control.

*Cryogenic mill*: It is used to grind products that cannot be ground by conventional methods. Cryogenic grinding is especially useful when contamination and analyte loss are critical concerns. Liquid nitrogen is normally used as cooling agent. The low temperature converts the sample into a brittle material facilitating the grinding process and protecting it from the atmospheric oxygen.[5] For this grinding process, a mass of 1.5 g sample was frozen with liquid nitrogen and ground during 6 min in three alternated grinding-freezing steps.

*Mortar and pestle*: It is a traditional grinding process in which the reduction of the particles size is achieved by squashing the sample with a pestle. It is applicable to small volumes of hard and abrasive materials. In this case, only the mass necessary for study was ground without any temperature control.

The effects of sample mass, particle sizes, and different grinding processes on sample homogeneity and representativity were investigated. Samples from different grinding processes were sieved by using screens with different meshes (32 and 42 mesh, i.e., 0.35 and 0.50 mm), and different particle sizes were obtained: $\phi > 0.50$ mm (fraction 1), $0.35 < \phi < 0.50$ mm (fraction 2), and $\phi < 0.35$ mm (fraction 3).

Samples sieved were acid digested using a microwave-assisted procedure, and the final solutions were analyzed by ICP OES. For the digestion procedure, sample masses of either 50.0 or 250.0 mg were directly weighed in a Teflon-PFA digestion vessel. Aliquots of 3 mL of $H_2O_2$ 30% v/v and 5 mL of $HNO_3$ 2 mol $L^{-1}$ were added, and the vessels were placed on the turntable. The microwave oven was operated according to the parameters listed in Table 2. The procedure was carried out in triplicate for each sieved sample in order to obtain statistically meaningful results. The resulting solutions were diluted to either 50.0 mL (250.0 mg) or 10.0 mL (50.0 mg) with deionized water, and analytes were determined by ICP OES.

**TABLE 2** Heating Program used in Microwave-Assisted Acid Digestion

| Step | Time (min) | Applied power (W) | Temperature (°C) |
|---|---|---|---|
| 1 | 1.5 | 250 | 120 |
| 2 | 1.5 | 0 | 120 |
| 3 | 5.0 | 550 | 210 |
| 5 | 5.0 | 700 | 210 |
| Ventilation | 5.0 | — | — |

## RESULTS AND DISCUSSION

### Effects of Sample Mass and Homogeneity on Representativity

Homogeneity is one of the most critical factors affecting sample representativity. Important analytical parameters such as accuracy and precision can be significantly affected when analyzing heterogeneous materials without proper sample treatment.[6,9] Figure 1 shows the breakfast cereal samples without any treatment and after homogenization using three different procedures. The differences of homogeneity among the samples are visually clear. The fractions not ground (original sample) and mortar and pestle ground are heterogeneous whereas the ones submitted to either knives or cryogenic grinding processes are visually homogeneous. The effects of those differences on precision are presented in Table 3. Considering both sample masses adopted, (i.e., 50.0 and 250.0 mg), the sample submitted to the cryogenic grinding process (the most homogeneous) is the one presenting the best precision for most elements. On the other hand, the original sample (the most heterogeneous) presents the worst precision for all elements studied. Whereas the cryogenic grinding process presented results as precise as 0.031% for Na, for example, the fraction not ground presented variance as high as 118% for the same element ($m = 250.0$ mg, $n = 3$).

Sample mass is another important parameter related to sample representativity.[11] As demonstrated before by using macroscopic models,[7,8] the sample mass can affect directly the precision. It can be seen in Table 3 that for the most homogeneous sample fractions (i.e., cryogenic and knives ground samples), great sample masses led to lower variance. Except for Fe in the cryogenic ground sample, smaller variations in the results were observed for all elements when a 250.0 mg sample aliquot was adopted. The results for Fe may be explained by a possible contamination in one of the cryogenic ground sample replicates. It should be mentioned that the internal bar and the stoppers in the sample polycarbonate recipient used in the cryogenic mill are made of stainless steel. These results confirm previous data and reinforce the effects of sample homogeneity[9] and sample mass[7,8,11] on precision.

It is interesting to note that the effect of sample mass on precision is less pronounced as sample homogeneity decreases. The sample fraction submitted to the mortar and pestle grinding process presented more precise results for a 250.0 mg sample aliquot only for four elements, (i.e., Ca, K, Mg, and P). For the original not-ground sample, only two elements (i.e., Mg and P), presented more precise results for a greater sample mass (Table 3). Because large particles in heterogeneous samples can have masses as great as 50.0 mg, small particles are systematically chosen for all replicates, and more precise results are obtained when working with 50.0 mg sample fractions. However, the accuracy of results obtained with those fractions can be compromised as they do not properly represent the sample.

### Effect of Particle Sizes on Representativity

Another factor of paramount importance to sample representativity is the particle size distribution. Aggregation of large particles can occur during the sampling step compromising the sample representativity.[7,8] Thus, the smaller is the particle size in a sample, the larger is the number of particles necessary to reach a given mass and, consequently, the sample aliquot is more representative.

To evaluate the influence of particle sizes on sample representativity, the sample fractions submitted to the grinding processes described above were

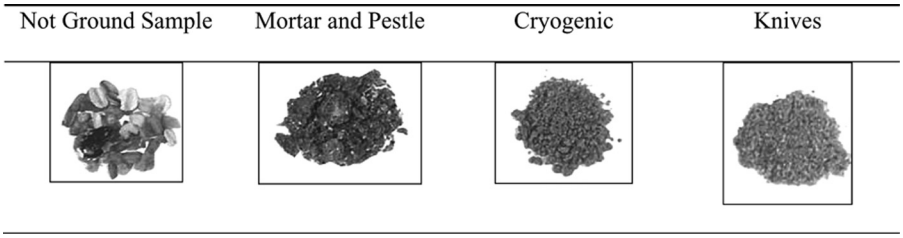


**FIGURE 1** Breakfast cereal sample not ground or submitted to mortar/pestle, cryogenic, and knives grinding processes, respectively.

**TABLE 3** Effects of Sample Mass and Homogeneity on Precision

| Sample | Sample mass (mg) | Concentration (mg kg$^{-1}$)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ca | Fe | K | Mg | Na | P | S | Zn |
| Original | 50.0 | 226±49 | 49.8±11.6 | 3790±970 | 517±354 | 3990±1730 | 1810±1270 | 850±404 | 47.7±15.8 |
| | 250.0 | 299±76 | 185±147 | 3740±2440 | 542±144 | 2480±2920 | 1700±510 | 914±470 | 165±132 |
| Mortar and pestle | 50.0 | 303±46 | 121±10 | 2540±390 | 427±32 | 4460±260 | 1360±80 | 880±26 | 116±5 |
| | 250.0 | 260±12 | 143±14 | 3020±210 | 472±7 | 4310±260 | 1500±50 | 989±65 | 121±6 |
| Knives | 50.0 | 308±31 | 134±61 | 2820±240 | 508±62 | 3130±300 | 1700±200 | 1190±100 | 101±14 |
| | 250.0 | 292±7 | 156±20 | 2640±1 | 644±13 | 3640±20 | 1960±10 | 1130±30 | 141±4 |
| Cryogenic | 50.0 | 354±18 | 178±16 | 2530±80 | 676±44 | 3490±180 | 2150±140 | 1150±40 | 137±19 |
| | 250.0 | 323±3 | 116±19 | 3130±20 | 556±4 | 3260±1 | 1750±20 | 1020±2 | 111±1 |

[a] Values reported as mean ±1 standard deviation (n = 3).

classified according to their particle sizes. Two screens with meshes of different diameters (0.35 or 0.50 mm) were used to separate the particles. The results can be used to explain the different standard deviation values found for each sample fraction.

The fraction submitted to the cryogenic grinding process presented particles with diameters, on average, smaller than 0.35 mm. On the other hand, the fractions not ground or submitted to the mortar/pestle process presented particles, on average, greater than 0.50 mm. For sample aliquots submitted to the knives grinding process, it was possible to obtain three different fractions: (1) particles $\phi > 0.50$ mm, (2) particles $0.35 < \phi < 0.50$ mm, and (3) particles $\phi < 0.35$ mm.

Data in Table 4 show the significant effect of the particles size on precision. As observed in previous works,[7,8,10] the smaller the particle sizes, the more representative is the sample aliquot and the higher is the precision. Smaller variation was observed for most elements when sample fractions presenting particle sizes smaller than 0.50 mm were analyzed. It can be observed in Table 4 that whereas fraction 3 presented relative standard deviation values smaller than 2.6% for 6 of 8 elements, fraction 1 presented values as high as 15% for most elements.

An interesting observation was that some elements known as macroelements, such as Ca, Mg, P, and S, were found in higher concentrations in fraction 1, and others such as Fe and Zn, considered microelements, were found in higher concentrations in fractions 2 and 3. This could be an indication that the macroelements are concentrated in some hard, fibrous components whereas the microelements would be present in higher concentrations in some other softer, easily ground components of the sample. However, these comments cannot be generalized for other types of samples.

## Representativity Affecting Accuracy

A common misunderstanding in freshmen analytical chemistry classes is related to the concepts of precision and accuracy. Students frequently associate precise results with accuracy and have difficulty visualizing the difference between both. Based on

**TABLE 4** Effects of Particle Size on Precision

| Fraction[a] | Particle sizes ($\phi$)(mm) | % mass[b] | Concentration (mg kg$^{-1}$)[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ca | Fe | K | Mg | Na | P | S | Zn |
| 1 | $>0.50$ | 54.2 | 352±15 | 167±19 | 2860±120 | 823±86 | 3470±290 | 2570±260 | 1430±210 | 123±1 |
| 2 | $0.35<\phi<0.50$ | 22.7 | 305±19 | 188±12 | 2550±100 | 675±16 | 4080±130 | 2100±30 | 1370±200 | 151±14 |
| 3 | $<0.35$ | 23.1 | 231±6 | 288±23 | 2430±60 | 504±13 | 3760±80 | 1930±40 | 1290±160 | 147±3 |

[a] Sample mass 50.0 mg.
[b] Based on knives ground sample.
[c] Values reported as mean±1 standard deviation (n = 3).

the results presented above, the most homogeneous sample fractions were the ones submitted to either cryogenic or knives grinding processes. A sample mass of 250.0 mg and particles smaller than 0.50 mm allowed for more precise results for all elements studied. In spite of the precise results presented for the knives ground sample, the values obtained for K are smaller than the ones obtained for the other 250.0 mg sample fractions (Table 3). Despite the suitable precision (RSD = 0.040%), K value in the knives ground sample was more than 15% smaller when compared with the values determined for the cryogenic ground sample, for example. This difference can be explained by the fact that during the sample homogenization using the knives grinding process, the raisins present in the sample did not pass through the sieves, remaining retained inside the mill. Since raisins are known to contain high concentrations of K, the low values obtained for this element can be directly related to losses during the grinding process. Thus, the resulting sample fraction did not fully represent the original sample and, despite the low variance, the results could not be considered accurate.

Another factor that can affect accuracy is sample contamination. From Na values presented in Table 3, can be seen a considerable difference between the sample fraction submitted to the mortar and pestle grinding process and the others. A 32% higher Na concentration is obtained for that fraction when compared with the cryogenic ground sample, for example. Those Na high values can be explained by contamination due to the manual grinding in the mortar and pestle sample homogenization. In this case, results for Na in the mortar and pestle ground sample are neither precise nor accurate.

As discussed before, particle size distribution can affect sample representativity and, consequently, accuracy. One example of this fact can be observed from the P results for the 250.0 mg sample fractions presented in Table 3. It can be seen that values presented for the knives ground sample are higher than for the other sample fractions. After separating knives ground sample into fractions 1, 2, and 3, it was observed that fraction 1 presented masses approximately 2.4 times greater than those of the other fractions (Table 4). Because aggregation of large particles can happen during the sampling of heterogeneous samples,[7,8] and P is most concentrated in fraction 1 (Table 4), the results for this element in the knives ground fraction would be overestimated. When this aspect is corrected (i.e., P concentration in fraction 1 is divided by 2.4 and a new average is calculated from fractions 2, 3, and the corrected value of fraction 1), the new value did not differ statistically from the cryogenic ground sample at a 95% confidence level (Table 5).

**TABLE 5** **Phosphorus Concentrations in Breakfast Cereal Sample Submitted to Either Cryogenic or Knives Grinding Processes and Determined by ICP OES (Mean ± Confidence Interval, n = 3, p = 0.05, m = 250.0 mg)**

| Grinding process | Concentration (mg $kg^{-1}$ Phosphorus) |
|---|---|
| Cryogenic | 1750±50 |
| Knives | 1960±20 |
| Knives corrected | 1700±20 |

## CONCLUSIONS

Sampling exerts a significant effect on analytical procedures. The most important parameter regarding the sampling step is sample representativity. The proposed experiment allows the introduction of important concepts related to sample representativity to freshmen students in analytical chemistry laboratory courses. The effects of some fundamental parameters such as sample homogeneity, particle sizes, and sample mass on precision and accuracy can be exploited by using real samples. Sample grinding and standard solutions preparation can be made in advance, and students can perform the sample digestion and ICP OES analysis in a 4-h lab class. A single, element experiment using a more trivial analytical method such as flame atomic absorption spectrometry can be adopted for shorter classes or less equipped labs. Data can be used to complement observations from macroscopic-model exercises and also to reinforce theoretical concepts in analytical chemistry courses.

## ACKNOWLEDGMENTS

The authors are grateful for the financial support and fellowships provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico

e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

## REFERENCES


1. Kellner, R.; Mermet, J. M.; Otto, M.; Widmer, H. M.; Eds. *Analytical Chemistry*, 1st ed. Wiley-VCH: Weinheim, Germany, 1998; 941p.
2. Evans, E. H.; Day, J. A.; Price, W. J.; Smith, C. M. M.; Sutton, K.; Tyson, J. F. Atomic spectrometry update. Advances in atomic emission, absorption and fluorescence spectrometry and related techniques. *J. Anal. At. Spectrom*. **2003**, *18*(7), 808–833.
3. Markert, B. Sample Preparation (Cleaning, Drying, Homogenization) for trace element analysis in plant matrices. *Sci. Total Environ.* **1995**, *176*(1–3), 45–61.
4. Skoog, D. A.; West, D. M.; Holler, F. J.; Crouch, S. R. *Fundamentals of Analytical Chemistry*; 8th ed. Thomson Brooks/Cole: Belmont, CA, U.S.A., 2004; 992p.
5. Krug, F. J., Ed. Métodos de Preparos de Amostras-Fundamentos Sobre Preparo de Amostras Orgânicas e Inorgânicas Para Anālise Elementar, 1st Ed. CENA/USP: Piracicaba, Brazil, 2008; 340p.
6. Gouveia, S. T.; Lopes, G. S.; Fatibello-Filho, O.; Nogueira, A. R. A.; Nóbrega, J. A. Homogenization of Breakfast Cereal Using Cryogenic Grinding. *J. Food Eng.* **2002**, *51*(1), 59–63.
7. Vitt, J. E.; Engstrom, R. C. Effect of Sample Size on Sampling Error: An Experiment for Introductory Analytical Chemistry. *J. Chem. Educ.* **1999**, *76*(1), 99–100.
8. Ross, M. R. A Classroom Exercise in Sampling Technique. *J. Chem. Educ.* **2000**, *77*(8), 1015–1016.
9. Hartman, J. R.; Bacon, D. W.; Wolsey, W. C. An In-class experiment to illustrate the importance of sampling techniques and statistical analysis of data to quantitative analysis students. *J. Chem. Educ.* **2000**, *77*, 1017–1018.
10. Dunn, J. G.; Phillips, D. N.; van Bronswijk, W. An exercise to illustrate the importance of sample preparation in chemical analysis. *J. Chem. Educ.* **1997**, *74*, 1188–1190.
11. Guy, R. D.; Ramaley, L.; Wentzell, P. D. An Experiment in the Sampling of Solids for Chemical Analysis. *J. Chem. Educ.* **1998**, *75*, 1028–1033.