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Introduction

> *Sample preparation remains the single most challenging aspect of chemical analysis*
>
> Mary Ellen P. McNally [1]

Samples arriving in the analytical laboratory usually cannot be applied directly to analytical instruments. After homogenization of the material, a more or less complex pre-treatment consisting of several steps is required for almost every sample before analysis. Each step is critical and can be a source of error and additional contamination. Often the used methods generate hazardous waste from solvents, chemicals, and consumables used. Most of the cost in chemical analysis is associated with the efforts in sample preparation procedures.

The goal of the sample preparation process is the extraction of the analytes from the incompatible matrix. Especially in trace analysis, the target analytes in low concentration are embedded in a high excess of a hard to eliminate matrix. The enrichment of the analytes to a suitable level of concentration for detection and quantification often also includes a necessary clean-up from the co-extracts. The sample preparation requires most of the time and the best skills in the analytical laboratory. Many of these tasks are still manual today. A recent survey revealed that two-thirds of the analysis time is spent on sample preparation [2]. Optimum and fit-for-purpose preparation methods for analytes in diverse matrices are subject to discussion in thousands of scientific publications every year. Google Scholar finds more than 2 million hits featuring specific sample preparations alone in the last 10 years! [3]. The demand for multi-analyte methods for larger groups of compounds with a potential occurrence in the same sample, targeted or non-targeted, like pesticides, mycotoxins, drugs, or personal care products, just to name a few areas, is increasingly addressed. The continuously growing number of analytes in different matrices is the main reason for steadily ongoing improvements.

These very first steps in the analytical workflow have the highest impact on the quality of the analytical data. Many laboratories use manual, time- and labor-intensive procedures. But the manual sample pre-processing is the biggest known source of error in the analytical sequence, no matter how precise and sensitive a mass spectrometer in the final step may be, it cannot correct for [4].

Automated Sample Preparation: Methods for GC-MS and LC-MS,
First Edition. Hans-Joachim Hübschmann.

Errors in analytical measurements are the random variability, expressed as the precision of the method, systematic bias affecting the trueness of results, and gross mistakes, the handling errors. Method validation assesses precision and trueness of a method, while human spurious errors, reported as the greatest source of errors, cannot be corrected in the course of analysis [5]. It is reported from the survey that operator and sample processing errors amount up to 50% of all known potential sources of error. Evidently, measures in this area improve analytical quality significantly. Here is the standardization of sample preparation procedures a general goal. The introduction of instrumental and integrated sample preparation workflows addresses these weak points in the sample processing sequence of the otherwise excellently validated procedures.

Standardization of analytical methods including sample preparations for different kinds of samples is available for all routine areas with validated procedures published as European standards (EN), the methods of the International Organization for Standardization (ISO), the Association of Official Analytical Chemists (now AOAC International), American Oil Chemists' Society (AOCS), the Food and Drug Administrations (FDA), the Environmental Protection Agencies (EPA), Pharmacopias, and similar organizations. In these established methods, sample preparation is often the rate-determining step for sample throughput and too often the error-prone part of the analytical method. In this context, standardization calls for automation, not vice versa. Automation reduces the quite normal human manual variation and mistakes in the sample processing using adequately configured robots for the standard workflows.

In chemical analysis, the instrumentation for chromatographic separation and detection, in particular with the use of mass spectrometry, reached an operational and performance level of high technical maturity. Sensitivity, selectivity, separation power and mass resolution, speed of analysis, and data processing were the major instrumental developments with significant enhancements in recent years. Barely exploited are features for a major step ahead for an instrumental and robotic sample processing. The obvious gap is the missing use of such amazing instrument specifications for a greener sample preparation at the front end. Much smaller sample sizes are possible to reach legally required quantitation limits today. The miniaturization and standardization with automated robotic workflows for the analytical sample processing significantly release from the human impact on data quality. This handbook about *Automated Sample Processing* focuses on the tools, modules, and workflows for the next level of a greener analytical chemistry.

1.1 A Perspective on Human Performance

In contrast to the notable improvement in instrument performance and reliability, there was not much focus in the past on the instrumental integration of the traditional manually performed sample preparation workflows, knowing the significant impact of human error on reliable and true analysis results. A general industry view of human performance and root cause of events monitored is shown in Figure 1.1.

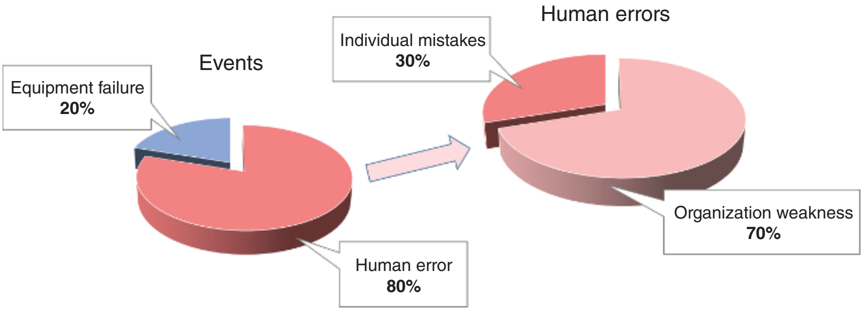

Figure 1.1 Human performance in analytical laboratories. Source: Adapted from U.S. Department of Energy [6].

Human error is not random. Mistakes are systematically connected to features of people's tools, the tasks they perform, and the operating environment in which they work [6]. About 80% of all events are attributed to human error. Further broken down, 70% are related to organizational weaknesses caused by humans in the past and 30% are directly related to human mistakes in the manual workflow.

Survey results confirm dramatically the sources of error in chemical analysis, illustrated in Figure 1.2 with the size of the square areas representing their impact. The major source of error is seen in such manual sample preparation steps with about 30% of all the potential causes. On top, operator-generated error and variation are estimated here to contribute to an additional 19%. The introduction of instrumental automated sample preparation workflows hence is expected to reduce such well-known human errors by half in routine analysis methods [5].

Samples to be analyzed usually require an often multifaceted preparation over several steps for extraction and concentration before application to an analytical instrument for analyte detection. More than 65% of the respondents to the recent survey reported the use of three or more sample preparation steps for one sample. The rate of more complex sample preparation went up significantly from previous

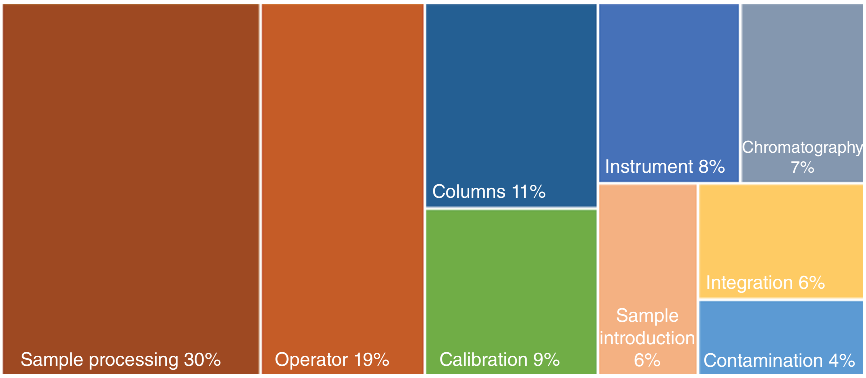

Figure 1.2 Survey results about sources of errors with impact in sample analysis (square areas in %). Source: Adapted from Majors [2].

years' responses, a number and apparent demand strongly increasing [7]. The degree of sample preparation depends mainly on the analytes, the physical status of the sample, the single or multi-compound approach, and finally the analytical method to be applied. It can cover a wide range from just dilute-and-shoot to multistep methods with extraction, concentration, and derivatization. The common goal is to reach just enough and fit-for-purpose sample clean-up to keep the analytical instrumentation in the validated status for large series of samples and reduce preventive maintenance downtime to the necessary. In the vast majority of analytical methods, the required sample preparation in the past and still today is manual. A large gap was left here, with only a few exceptions with some selected techniques, for the adaptation of analytical sample preparation methods on the instrument level. The solutions provided in this textbook address this notorious gap with the integration of robotic workflows for many routine tasks. A dedicated section covers automated turnkey solutions like the analysis of pesticides, off-odors, polyaromatic hydrocarbons (PAH), fatty acid methyl esters (FAMEs), mineral oil hydrocarbon contaminations (MOHs), or volatile organic compounds (VOCs), just to name a few that are presented in detail for reproduction.

The recent remarkable improvements in instrument sensitivity and selectivity, in particular in mass spectrometry using tandem instrumentation (MS/MS) and high mass resolution and accurate mass capabilities (HR/AM), greatly facilitated the miniaturization of such methods, allowing greener analytical chemistry with the reduction of sample sizes and solvent use. This sample volume reduction to the micro-scale opens up the way for compatible, instrument-top automation and further standardization of multi-methods. This welcome trend answers also the demand for improved data quality and higher sample throughput, especially in food, environmental, and pharmaceutical safety analyses.

Another important aspect and a strong improvement is seen in the hyphenation of instruments with workflows integrating the currently separated processes. The availability of online sample preparation, which finally includes the transfer and injection of the prepared extract to the analytical instrument, is today still the exception in analytical instrument design. Ongoing technical development starts covering some selected sample preparation procedures with benchtop or instrument top-mounted robotic preparation systems. The integrated software control for ease of use with just one sample acquisition sequence on screen is still a big but solvable gap to comply with operator demands and the required error-free operation. Several independent developments of integrated software control of the robotic sample processing systems with external devices and the hyphenated analytical instruments demonstrate successfully the benefits and feasibility.

And, even in light of the continuously increasing laboratory automation, a necessary practical remark at this point for the hands-on laboratory work, for safety and a green analytical chemistry: good laboratory practice dictates that all who handle solvents and chemicals should familiarize themselves with the compounds' material safety data sheets (MSDS) and manufacturer's recommendations for handling, use, storage, and disposal of the used chemicals.

References

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