PREVIEW
SAMPLE PREPARATION FUNDAMENTALS FOR CHROMATOGRAPHY

The Measure of Confidence

Agilent Technologies

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Preface

Since most samples encountered in a chromatography laboratory are not in a form to be directly placed into the analytical instrument, some form of preparation is required for nearly every sample. The sample preparation could be as simple as "dilute and shoot" or as complex as multistage sample handling. The analytical cycle represents all of the steps from the point of collection to the final analysis and data output. Although sample preparation is an important part of this analytical cycle, it doesn't always get the respect as does the separation and measurement instrumentation and the data handling aspects. Oftentimes, the task of sample preparation employs decades old technology that is often manual, time-consuming and uses a lot of glassware and other devices, and some older technologies use copious amounts of solvent that must eventually be disposed of, creating expense and safety issues. Because of the use of multiple sample preparation steps in an attempt to simplify and/or isolate the desired analytes from a complex matrix, errors tend to creep into the assay and analyte recoveries may suffer.

The purpose of this book is to outline some of the most popular sample preparation technologies in current use today. Since sample preparation technologies is represented by tens of possible sample manipulations (e.g. weighing, dissolution, extraction, trapping, etc.), I didn't set out to cover every single sample prep category. The book started out to be a small handbook like the popular *The LC Handbook: Guide to LC Columns and Method Development* (Publication Number 5990-7595EN) but soon blossomed into a 350+ page book covering many different sample preparation technologies. Since the book was written for Agilent Technologies, many of the methodologies covered are those within Agilent's chemistries portfolio, but for the sake of completeness, I have covered a number of technologies outside of Agilent's immediate areas of interest. Examples of applications are provided throughout the book and many of them are web-accessible.

Since this book is primarily designed for the chromatography laboratory and to keep the length reasonable, I had to confine my coverage to organic and biological sample preparation and thus inorganic sample prep, although important in chromatographic and obviously the spectroscopic analyses of many sample types, was omitted. Many of the techniques covered, however, such as ion exchange SPE, liquid-liquid extraction and microwave-assisted extraction are equally applicable to inorganic samples for further analysis by ion chromatography or for spectroscopic measurements.

I have written the book in a slightly different format than might be typical. After an introductory Chapter 1 on the sample prep process, in Chapter 2, I decided to tabulate most of the methodologies that will be covered in the remainder of the book. That way, the reader, rather than wading through all the various chapters, can get an overview of possible sample preparation methods that are most applicable to gases, liquids, suspensions, gels and solid materials. So the reader can get directly to the sample prep methodology that may suit his/her particular sample. In subsequent Chapters, rather than repeating all the information, I refer back to these tables. The book is organized by the flow of sample preparation process (sampling, transport, storage all the way up to sample filtration and, in some cases, sample introduction).

The Chapters are further organized by sample types so that gaseous samples are first (Chapter 6) followed by liquid samples (or samples put into a liquid form) (Chapters 7-12), solid samples (Chapter 14), biological samples (nucleic acids and proteins)(Chapter 15), biological fluids/tissues (Chapter 16) and then special topics such as sample prep for mass spectrometry, membrane applications, chemical scavengers, and derivatizations. Finally, Chapter 21 covers a new concept of "Just Enough" sample preparation that seems to be today's trend relying heavily on the increased use of hyphenated-chromatography/tandem mass spectrometry techniques. To aid the novice (and maybe some of the experts!) on the terminology associated with sample prep, the final Chapter 22 includes a Glossary.

I wish you good reading and hope that the material within provides you with a good foundation on how to best approach your sample preparation challenges.

Ronald E. Majors, Wilmington, DE

Chapter 1
Introduction

The major stages of an analytical process are depicted in **Figure 1.1**. The proper choice of the measurement technique is only one step in the development of a successful application. All of the steps leading up to the measurement are as important. The sampling and sample preparation process begins at the point of collection and extends to the measurement step. The proper collection of sample during the sampling process (called primary sampling) is the initial contact with the sample and it is imperative that this sample represents the entire lot being investigated. The storage, preservation and transport of this representative sample from the point of collection to the analytical laboratory must occur without any changes in the physical and chemical makeup of the original sample. The proper selection of the laboratory sample itself (termed secondary sampling) represents another challenge since the final sample used for analysis may be a tiny fraction of the original collected sample, yet serves as a subset. Finally, the sample preparation methodology necessary to convert the sample into a form suitable for the measurement step must also be performed without loss or any unplanned modification of the secondary sample. All of these pre-analysis steps can have a greater effect in the overall accuracy and reliability of the results than the measurement itself.

Figure 1.1

Sample Analysis Workflow Diagram

1
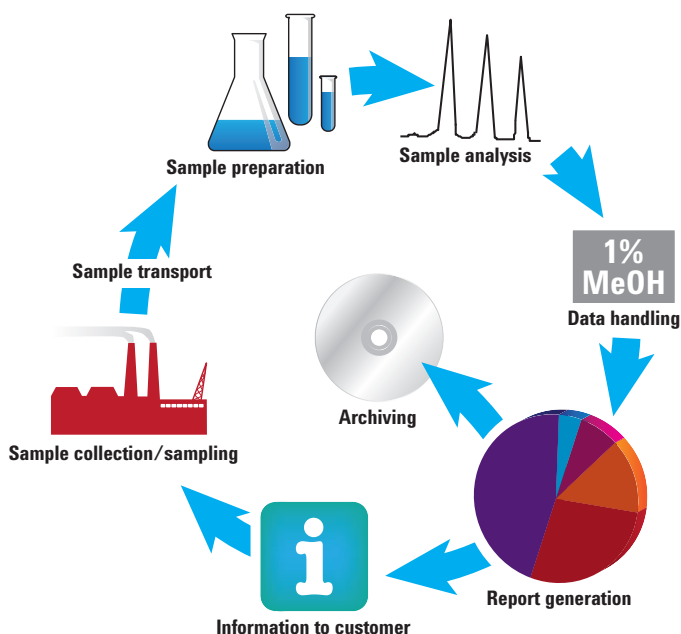
Sample preparation is an essential part of chromatographic and spectroscopic analyses. The process is intended to provide a representative, reproducible, and homogenous solution that is suitable for injection into the column for chromatographic analysis, or into an ICP-MS/atomic adsorption source, or into a cuvette or NMR tube for further characterization. The aim of sample preparation is to provide a sample aliquot that (a) is relatively free of interferences, (b) will not damage the column or instrument and (c) is compatible with the intended analytical method. In chromatography, the sample solvent should dissolve in the HPLC mobile phase or be injectable into a GC column without affecting sample retention or resolution, the stationary phase itself, and without interfering with detection. It is further desirable to concentrate the analytes and/or derivatize them for improved detection or better separation. In spectroscopy, the sample solvent should be free of particulates, compatible with the spectroscopic source, and be of the appropriate viscosity to flow into a nebulizer for on-line methods. Sometimes, depending on spectroscopic sensitivity, preconcentration is needed and chromatography or liquid-liquid extraction is sometimes used prior to introduction of the sample into the instrument.

Although many of the sample preparation protocols used in chromatography and spectroscopy are similar, it is beyond the scope of this book to address the various differences between sample preparation procedures for these diverse methods. Therefore, we will limit the topics in this handbook to the popular sample preparation methods for chromatographic analysis with emphasis on Liquid Chromatography (LC)/High Performance LC (HPLC)/Ultra HPLC (UHPLC), and Gas Chromatography (GC).

A more detailed depiction of the various operations in the analytical cycle is summarized in **Table 1.1**. If particular attention is not paid to all of these operations, sample integrity may be sacrificed and the analysis data affected, compromised, or rendered invalid. Steps 1-5, which include 1) sample collection, 2) storage and preservation, 3) sample transport, 4) preliminary processing and laboratory sampling, and 5) weighing or dilution, all form an important part of sample preparation. Although these steps in the chromatographic assay can have a critical effect on the accuracy, precision, and convenience of the final method, space limitations preclude us from addressing all of these areas in detail. Only steps 1 and 4 (sample collection and preliminary sample processing) will be briefly explained here. See References 1-4 for an explanation of steps 2, 3, and 5. The bulk of this book will be devoted mainly to Steps 6-9 of **Table 1.1**, which encompasses what is usually meant by sample pre-treatment or sample preparation ("sample prep").

Table 1.1

	Sample Pre-treatment Options	
Step	**Option**	**Comment**
1	Sample collection	Obtain representative sample using statistically valid processes.
2	Sample storage and preservation	Use appropriate inert, tightly-sealed containers; be especially careful with volatile, unstable, or reactive materials; stabilize samples, if necessary; biological samples may require refrigeration or freezing.
3	Sample transport	The act of transporting the sample from the point of collection to the laboratory can be an important step. Transportation conditions should maintain its integrity, samples should not have rough handling, be dropped, or be allowed to be exposed to the elements; the timing may be important for samples — undue delays may cause sample degradation as in step 2 above.
4	Preliminary sample processing	Sample must be in form for more efficient sample pre-treatment (e.g. drying, sieving, grinding, etc.); finer dispersed samples are easier to obtain representative sample and to dissolve or extract.
5	Weighing or volumetric dilution	Take necessary precautions for reactive, unstable, or biological materials; for dilution, use calibrated volumetric glassware.
6	Alternative sample processing methods	Solvent exchange, desalting, evaporation, freeze drying, etc.
7	Removal of particulates	Filtration, centrifugation, solid phase extraction.
8	Sample extraction	Method for liquid samples (Table 2.4) and solid samples (Tables 2.2 and 2.3)
9	Derivatization	Mainly to enhance analyte detection; sometimes used to improve separation, extra step in analytical cycle adds time, complexity, and potential loss of sample (See Chapter 20).

Whereas GC and HPLC are predominantly automated procedures, sample pre-treatment is often performed manually. As a result, sample pre-treatment can require more time for method development and routine analysis than is needed for the separation and data analysis (see **Figure 1.2**). Sample pre-treatment may include a large number of methodologies, as well as multiple operational steps, and can therefore be a challenging part of chromatographic method development.

Figure 1.2

Time Spent on Typical Chromatographic Analysis

- Data management (27%)
- Collection (6%)
- Analysis (6%)
- Sample processing (61%)
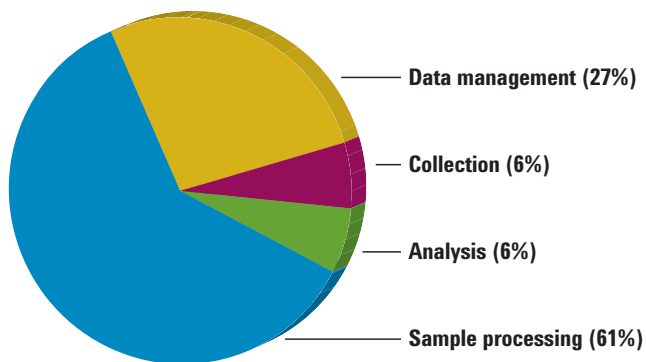
Data taken from Agilent Technologies survey

Finally, method precision and accuracy is frequently determined by the sample pre-treatment procedure (see **Figure 1.3**), including operations such as weighing and dilution. For all of these reasons, the development of a sample pre-treatment procedure deserves careful, advance planning.

Figure 1.3

Sources of Error Generated During Chromatographic Analysis

- Contamination (4%)
- Columns (11%)
- Operator (19%)
- Sample introduction (6%)
- Chromatography (7%)
- Integration (6%)
- Instrument (8%)
- Calibration (9%)
- Sample processing (30%)

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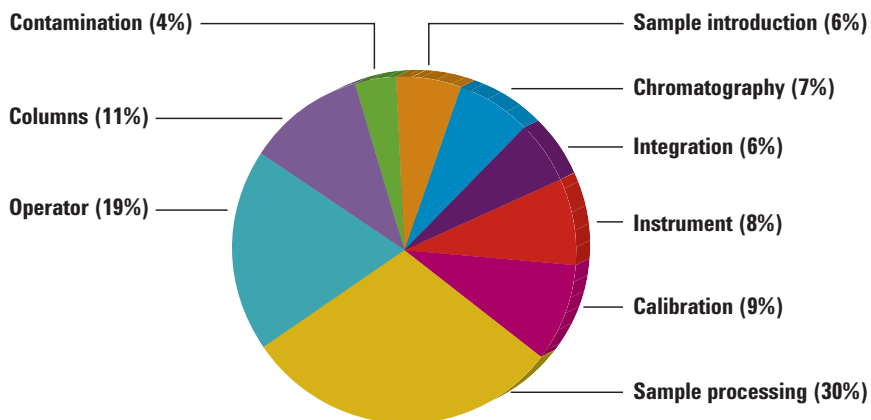
A sample pre-treatment procedure should provide quantitative recovery of analytes, involve a minimum number of steps, and (if possible) be easily automated. Quantitative (99+%) recovery of each analyte enhances sensitivity and assay precision, although this does not mean that all of the analyte present in the original sample must be included in the final injected sample. For example, in a given method, for a series of sample pre-treatment steps, aliquots of intermediate fractions may be used for further sample preparation or for an intermediate injection. If recovery is not 100%, the sample pre-treatment must be reproducible. The use of internal standards or standard addition are approaches to aid in better quantitation, when recovery is not complete. As implied in **Figure 1.3**, a smaller number of sample pre-treatment steps plus automation reduces the overall time and effort required, and decreases the opportunity for imprecision and accuracy errors by the analyst. Thus, depending on the chromatographic and detection selectivity available, one should attempt to use as few sample pre-treatment steps as possible. In other words, sufficient sample preparation should be performed to meet the goals of the analysis, dependent on selectivity available in other parts of the analysis system (e.g. chromatography, detection), sufficient sample preparation may entail multiple steps and techniques. This concept is thoroughly explained in Chapter 21.

Many sample preparation techniques have been automated, and usually appropriate instrumentation is commercially available. Approaches to automation vary from use of a robot to perform manual tasks to dedicated instruments optimized to perform a specific sample preparation technique. While automation can be expensive and elaborate, it is often desirable when large numbers of samples must be analyzed and the time or labor per sample is excessive. The decision to automate a sample pre-treatment procedure is often based on a cost justification, the availability of instrumentation to perform the task at hand, or in some cases, when operator safety is involved (i.e. to minimize exposure to toxic substances or other possible health hazards). Sample preparation instrumentation for automation will be briefly addressed here, but it is beyond the scope of this book to elaborate further on the details of commercial instrumentation. Refer to textbooks on the subject listed under references 5-7.

Table 1.2

As you embark on using the remainder of this book, there are many questions that you must consider before deciding which sample preparation technique may be the best for your particular sample. **Table 1.2** is a sample preparation worksheet that provides some guidance in consideration of the goals of your analytical method and sample preparation including analyte and matrix questions. Because there are so many terms associated with sample preparation, a Sample Preparation Glossary is provided in the Appendix of this book. For abbreviations and definitions that you may encounter, please refer to the Glossary.

Sample Preparation Worksheet		
Sample Preparation Questions	**Example/Considerations**	**Comments**
What is analytical measurement technique?	LC-UV, LC/MS, GC/MS, etc.	Final sample must be compatible with analytical technique.
What is your optimal analytical run time?	1 min, 10 min, .5 hour, longer	Sample prep time may exceed run time; can you batch samples?
What level of recovery is required to meet LOD/LOQ?	100% only, less than 100%	100% recovery is ideal, but the more sample prep steps you have, the greater opportunity for loss; even so, RSD may still be acceptable at lower recovery if "loss" is reproducible.
How do you plan to quantitate?	External standard or internal standards?	Are standards available? Can you find internal standard(s) that are resolved from analytes of interest? Do you need multiple internal standards?
What is your required accuracy and precision?	Consider both inter- and intra-day values	For trace levels (e.g. sub-ppb) RSDs may be greater than you expect; must determine recovery, precision and accuracy at levels expected in your samples (minimum of 3 levels).
What is the sample matrix?	Organic, biological, inorganic, solid, semi-solid, liquid, gel, gas, etc.	Must choose sample prep technique that can selectively remove analytes of interest from the matrix.
How much do you know about the sample matrix?	Oil-based vs. aqueous-based, high salt content, volatile, unstable; is the sample matrix polar or non-polar, ionic, ionizable?	Must begin early to think about sample prep technique to best differentiate the properties of your analyte and matrix.

(Continued)

Sample Preparation Worksheet

Sample Preparation Questions	Example/Considerations	Comments
What is the sample volume/mass?	Microliter vs. liter, mg vs kg, etc.	Must have equipment and glassware available to handle size of sample required.
What key interferences are endogenous to the sample?	Are interferences more like matrix or like your analytes?	May require more than one sample prep technique for cleanup of interferences that are similar to your analytes of interest.
What functional groups on your matrix, interferences and analyte(s) of interest may influence choice of sample prep technique?	Influences solubility, polarity, ionization states (pKa)	Often don't know the actual structures of matrix and interferences to help make rational decision.
What else is already known about analyte(s) itself?	Water-octanol partition coefficients, concentration range, chemical structure	May allow you to direct your attention to capturing the analyte itself and rely upon the chromatography and/or detection step(s) to resolve it from any co-extracted interferences and matrix components.
What is level of interference removal required for analysis?	Depends on selectivity of chromatography separation and detection	Must avoid ion suppression/enhancement effects in LC/MS (and MS-MS); for UV and less selective detectors, and other, must have better sample prep and chromatography selectivity.
What sample pre-treatment steps may be required?	Dilution, clarification, filtration, pH adjustment, etc.	May be required for best overall selectivity, but each additional step can lead to analyte loss and affect accuracy/precision.
Is a concentration step required for optimal analysis?	Solvent evaporation, purge and trap, etc.	Concentration steps add time to analysis but may be required to meet LOC/LOQ.
What solvent should the analyte(s) be in for optimal analysis?	Avoid solvents that may cause UV interference, MS ion suppression, GC stationary phase compatibility, or non-volatile	Choice of solvents for sample prep final step may be limited; can always evaporate to dryness and re-dissolve in compatible solvent; increases time and number of steps.
What resources are available for method development and routine analysis?	High sample loads may require some level of automation; do you have the right sample prep tools available in your lab?	Sample prep may require more personnel since it is often more labor intensive and time consuming than the analytical measurement.

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References

1. Keith, L.H. (Ed.) *Principles of Environmental Sampling (ACS Professional Reference Book)*, 2nd Ed., American Chemical Society, Washington, DC, **1996**, ISBN-10: 0841231524 and ISBN-13: 978-0841231528.

2. Pawliszyn, Janusz, (Ed.) *Comprehensive Sampling and Sample Preparation: Analytical Techniques for Scientists*, Academic Press, Waltham, MA, **2012**, ISBN-10: 0123813735 and ISBN-13: 978-0123813732.

3. Svarc-Gajic, Jaroslava (Ed.) *Sampling and Sample Preparation in Analytical Chemistry, (Chemistry Research and Applications: Chemical Engineering Methods and Technology)*, Nova Science Pub Inc., Hauppauge, NY, **2011**, ISBN-10: 1621002691 and ISBN-13: 978-1621002697.

4. Conklin, Alfred R., Jr. *Field Sampling: Principle and Practices in Environmental Analysis*, Marcel-Dekker, New York, **2004**.

5. Moldoveanu, S.C.; David, V. *Sample Preparation in Chromatography (J. Chromatography Library)*, Elsevier Science, San Diego, CA, **2002**.

6. Wells, D.A. *High-Throughput Bioanalytical Sample Preparation: Methods and Automation Strategies*, Elsevier Science, San Diego, CA, **2003**, ISBN-10:044451029X and ISBN-13: 978-0444510297.

7. Nickerson, B. (Ed.) *Sample Preparation of Pharmaceutical Dosage Forms: Challenges and Strategies for Sample Preparation and Extraction*, Springer, New York, **2011**, ISBN-10:1441996303 and ISBN-13:978-1441996305.

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Types of Samples and Overview of Approaches to Processing

Sample matrices can be broadly classified as organic, biological or inorganic, and may be further subdivided into solids, semi-solids (including creams, gels, suspensions, colloids), liquids, and gases. For nearly every matrix, some form of sample pre-treatment, even if it is just simple dilution, will be required prior to chromatographic analysis.

Gaseous samples usually are analyzed by gas chromatography, rather than HPLC. Techniques such as canister collection, direct sampling via sample loops, headspace sampling and purge and trap are used to collect and inject gases. In Chapter 6, we will briefly cover those sampling/sample prep techniques primarily used for gas samples. **Table 2.1** provides an overview of typical sampling, sample introduction, and sample preparation procedures used for gaseous volatile samples.

Table 2.1

Typical Sampling and Sample Pre-treatment Methods for Gaseous Samples		
Method of Sample Pre-treatment	**Principles of Technique**	**Comments**
Grab Sampling	Gaseous sample is pulled into an evacuated glass, metal bulb or canister, or by a syringe; gas can also be pumped into plastic bag or other inert container.	Used mostly for volatile compounds in air; samples are returned to laboratory and analytes are isolated and concentrated by cold trapping techniques.
Solid Phase Trapping	Gaseous sample passed through tube packed with adsorbent (e.g. silica gel, activated carbon); trapped analytes are eluted with strong solvent.	Used for semivolatile organic compounds in air; control of gas flow rate is critical for trapping efficiency; watch for aerosol formation, adsorbent overloading, and irreversible adsorption of reactive analytes; popular sorbents include silica gel, alumina, porous polymers (Tenax, polyurethane foams), or carbon; chemical or physical complexing reagents may be useful to improve trapping efficiency.
Liquid Trapping	Gaseous sample is bubbled through solution that is a good solvent for analytes; analyte has higher affinity for solvent than it does for gas.	Flow rate should be low enough so as not to create foams or aerosols; complexing agents may be added to solvent to aid trapping; temperature can be lowered for very volatile species; sometimes process IS called "impinging."

(Continued)

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Table 2.1 (*Continued*)

Typical Sampling and Sample Pre-treatment Methods for Gaseous Samples

Method of Sample Pre-treatment	Principles of Technique	Comments
Headspace Sampling	Sample (solid or liquid) is placed in a closed, thermostated glass vial until equilibrium is established; at equilibrium, analytes partition themselves between a gas phase and the solid (or liquid) phase at a constant ratio; gas phase is sampled and injected into GC for analysis.	Used primarily for determination of trace concentrations of volatile substances in samples difficult to handle by conventional GC techniques; sensitivity can be increased by heating (<100 °C), salting out, adjusting pH, and other means to shift equilibrium; sometimes water or solvent is added to aid in sample dispersion and/or to free organics from the matrix, especially for soils and sediments; can be manual or automated.
Purge and Trap (Dynamic Headspace)	Sample (solid or liquid) is placed in closed, thermostated container and the headspace vapors are continually removed by means of inert gas flow with subsequent trapping of sample components by solid phase extraction or cold trapping; then thermally desorbed into GC injection port (thermal desorption).	Used when analytes are too low in concentration or have unfavorable partition coefficients in static headspace (HS) sampling (sometimes called gas phase stripping) can provide more sensitivity than static HS by accumulating the volatiles until concentration is sufficiently built up for thermal desorption and GC analysis; can be manual or automated.
Thermal Desorption	Used in conjunction with purge and trap and solid phase microextraction to concentrate volatile analytes; sorbent is rapidly heated to transfer concentrated analytes to GC by purge gas.	Typical adsorbent resins include Tenax TA, glass beads, Carbosieve, Carboxen, and Carbotrap. Sorbent choice based on specificity, breakthrough volume, water affinity, bed volume, and range of organics sorbed/desorbed from resin; can be cyrogenically cooled to trap volatile organics.
Direct Thermal Extraction	A form of dynamic headspace, but the sample is heated (controlled) to much higher temperatures, up to 350 °C.	System must be constructed of fused quartz or fused silica so that extracted analytes do not react with hot metal surfaces; system cold spots should be avoided; used primarily for semi-volatile compounds.
Pyrolysis	Non-volatile large molecule sample (e.g. polymer, plant fiber) is thermally degraded to cleave linkages and produce smaller, more volatile molecules that are swept into GC or to adsorbent trap (cyrogenic) for separation and identification.	Degradations often have defined mechanisms and sample may break apart in a predictable manner; can lead to structural information about starting compound or provide "fingerprints" for comparative profiles; pyrolysis can be performed in inert or reactive atmosphere.
Solid Phase Microextraction (SPME)	Fused silica fiber coated with polymeric stationary phase is placed in headspace above sample or directly into liquid sample; analytes diffuse and partition/adsorb onto stationary phase; analytes are thermally desorbed by placing fiber into GC injection port or displaced by means of a liquid to a column for HPLC analysis.	SPME is an equilibrium sampling method and can be used for gases, solids (Headspace) and liquids (direct); various polymer coating formulations available (e.g. polydimethylsiloxane (PDMS), polyacrylate, Carbowax-divinylbenzene, Carboxen-PDMS); can work with small sample sizes, is field transportable, and uses no organic solvent; very volatile analytes can sometimes be lost and quantitation is problematic.

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Volatile analytes that are labile, thermally unstable, or prone to adsorb to metal surfaces in the vapor state are sometimes better handled by HPLC. Trapping is required to analyze gaseous samples by HPLC. The gas sample is either (a) passed through a solid support and subsequently eluted with a solubilizing liquid, or (b) bubbled through a liquid that traps the analyte(s). An example of the HPLC analysis of a gaseous sample is the American Society for Testing Materials (ASTM) Method D5197-03 and United States Environmental Protection Agency Method TO-11 for volatile aldehydes and ketones[1, 2]. In this example, an air sample is passed through an adsorbent trap coated with 2,4-dinitrophenylhydrazine, which quantitatively converts aldehydes and ketones into 2,4-dinitrophenylhydrazones. The hydrazones are then eluted with acetonitrile and separated by reversed-phase HPLC.

Sample preparation for solid samples can often be more demanding. Samples that are solid (or semi-solid) must usually be put into a liquid form – unless the volatile portion only is of interest and then headspace, purge and trap, or thermal desorption techniques (covered in Chapter 6) are used to isolate, and perhaps concentrate, that portion of the sample. In some cases, the sample is easily dissolved and is then ready for injection or further pre-treatment. In other cases, the sample matrix may be insoluble in common solvents, and the analytes must be extracted from the solid matrix. There are also cases where the analytes are not easily removable from an insoluble matrix because of inclusion or adsorption. If the solvent-extractable portion of a solid sample is of interest, then techniques such as liquid-solid extraction, supercritical fluid extraction, microwave-assisted extraction, Soxhlet extraction, or pressurized fluid extraction can be used (see Chapter 14). Here, the solid material is exposed to a solublizing liquid or supercritical fluid (usually carbon dioxide, often doped with a polar solvent such as methanol), sometimes with added heat and/or pressure. Sample components soluble in the liquid eventually are totally or partially leached out of the sample. Obviously, the more porous the sample and the more finely divided the solid sample, the easier it is to extract components.

If the entire solid sample is to be analyzed, more drastic dissolution techniques or stronger solvents may be required. For example, a rock sample or metal may require digestion with a strong acid to completely solubilize it and then the liquified sample further treated to isolate components of interest. **Table 2.2** lists some traditional methods for the recovery of analytes from solid samples, while **Table 2.3** describes additional recent procedures. Once analytes have been quantitatively extracted from a solid sample, the resulting liquid fraction can either be injected directly into the HPLC or GC instrument, or subjected to further pre-treatment. Chapter 14 provides more details on the extraction of solid samples while Chapter 16 provides information on the extraction of solid- and semi-solid-biological samples, such as tissue.

Table 2.2

Traditional Methods for Sample Preparation of Solid Samples		
Method of Sample Pre-treatment	**Principles of Technique**	**Comments**
Solid-Liquid Extraction	Sample is placed in closed container and solvent is added that dissolves/extracts/leaches the analyte of interest; solution is separated from solid by filtration (sometimes called "shake/filter" method).	Solvent is sometimes boiled or refluxed to improve solubility; sample is in finely-divided state to aid leaching process; sample can be shaken manually or automatically; sample is filtered, decanted, or centrifuged to separate from insoluble solid.
Soxhlet Extraction	Sample is placed in disposable porous container (thimble); constantly refluxing fresh solvent flows through the thimble and dissolves analytes that are continuously collected in a boiling flask (see Chapter 14).	Extraction occurs in pure solvent; sample must be stable at boiling point of solvent; slow but extraction is carried out unattended until complete; inexpensive; best for freely flowing powders; excellent recoveries (used as standard to which other solid extraction methods are compared).
Homogenization	Sample is placed in a blender or a mechanical homogenizer, solvent is added, and sample is homogenized to a finely divided state; solvent is removed for further workup.	Used for plant and animal tissue, food, environmental samples; organic or aqueous solvent can be used; dry ice or diatomaceous earth can be added to make sample flow more freely; small dispersed sample promotes more efficient extraction.
Sonication	Use of ultrasound to create vigorous agitation at the surface of a finely divided solid material; direct method: uses a specially designed inert acoustical tool (horn or probe = sonotrode) placed in sample-solvent mixture; indirect method: sample container is immersed in ultrasonic bath with solvent and subject to ultrasonic radiation.	Dissolution is aided by ultrasonic process; heat can be added to increase rate of extraction; safe; rapid; best for coarse, granular materials; for indirect method, multiple samples can be done simultaneously; efficient contact with solvent.
Dissolution	Sample is treated with dissolving solvent and taken directly into solution with or without chemical change.	Inorganic solids (e.g. minerals, metals) may require acid or base digestion to completely dissolve; organic samples can often be dissolved directly in solvent; biological samples may not fully dissolve; for many sample types, filtration may be required after dissolution.

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Compared to gases or solids, liquid samples are much easier to prepare for chromatographic analysis.

Table 2.3

Modern Extraction Methods for Solid Samples

Method of Sample Pre-treatment	Principles of Technique	Comments
Pressurized Fluid Extraction (PFE)/Accelerated Solvent Extraction (ASE)	Sample is placed in a sealed container and heated to above its boiling point causing, pressure in vessel to rise; extracted sample is removed and transferred to vial for further treatment.	Greatly increases speed of liquid-solid extraction process; may be automated; vessel must withstand high pressure; extracted sample is diluted and requires further concentration; safety provisions are required because of overpressured, high temperature solvents.
Automated Soxhlet Extraction	A combination of hot solvent leaching and Soxhlet extraction; sample in thimble is first immersed in boiling solvent, then thimble is raised for conventional Soxhlet extraction/rinsing with solvent refluxing and finally concentration.	Semi-automated and automated versions available; uses less solvent than traditional Soxhlet, solvent is recovered for possible reuse; decreased extraction time due to two-step process.
Supercritical Fluid (SF) Extraction	Sample is placed in flow-through container and supercritical fluid (e.g. CO_2) is passed through sample; after depressurization, extracted analyte is collected in solvent or trapped on adsorbent followed by desorption by rinsing with solvent.	Automated and manual versions available; to affect "polarity" of SF fluid, density (vary temperature and/or pressure) can be varied and solvent modifiers added; collected sample is usually concentrated and relatively contaminant-free because CO_2 volatilizes after extraction; matrix affects extraction process; thus method development may take longer than other modern methods.
Microwave-Assisted Extraction	Sample is placed in a solvent in an open or closed container and contents heated by microwave energy causing a temperature rise and extraction of analyte.	Extraction solvent can range from microwave absorbing (MA) to non-microwave absorbing (NMA); in MA case, sample is placed in high pressure container and heated well above its boiling point as in PFE/ASE; in NMA case, microwave absorbing device placed container so solvent is indirectly heated; safety provisions are required with organic solvents in microwave oven (MA/NMA) and high pressures of MA example.

(Continued)

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Modern Extraction Methods for Solid Samples

Method of Sample Pre-treatment	Principles of Technique	Comments
Gas Phase Extraction	After equilibrium, analytes partition themselves between a gas phase and the solid phase at a constant ratio; static headspace: volatiles sampled above solid; dynamic headspace (purge & trap): volatiles sampled by continuously purging headspace above sample with inert gas and trapped on a solid medium, then thermally desorbed into GC; membrane can be used as interface between sample and flowing gas stream for added specificity.	Headspace techniques used for volatile analytes in solid samples; heat (usually <100 °C) can be applied to sample to speed up volatilization process; sometimes water or solvent is added to aid in sample dispersion and/or to free organics from the matrix, esp. for soils and sediments; both static and dynamic headspace techniques have been automated; dynamic techniques are more sensitive; microwaves have been used for heating.
Matrix Solid Phase Dispersion (MSPD)	Technique uses bonded phase supports as an abrasive to produce disruption of sample matrix architecture and as a "bound" solvent to aid complete sample disruption during the sample blending process.	Solid or viscous sample (approx. 0.5 g) is placed in mortar with about 2 g of SPE sorbent (e.g. C18) and is blended to homogenized mixture; sometimes solvent is added to aid extraction process; blend is transferred to column and analytes are eluted with solvent, sometimes to an SPE layer for further cleanup prior to injection; a "solid-solid" extraction process.

Many HPLC analyses are based on a "dilute and shoot" procedure, where the solubilized analyte concentration is reduced by dilution so as to not overload the column or saturate the detector. Often, liquid samples can be directly injected into a gas chromatograph and the volatile compounds separated and detected. In some cases, non-volatile compounds of the liquid sample may deposit in the injector, retention gap, or at the head of the column. Special GC conditions may be required to remove or eliminate this possibility. An overview of popular sample preparation methods for liquids and suspensions are listed in **Table 2.4**.

Table 2.4

Typical Sample Preparation Methods for Liquids and Suspensions

Methods of Sample Preparation	Principles of Technique	Comments
Solid Phase Extraction (SPE)	Similar to HPLC, sample is applied to, and liquid is passed through, a column packed solid phase that selectively removes analyte (or interferences); analyte can be eluted with strong solvent; in some cases, interferences are retained and analytes allowed to pass through solid phase unretained (Chapter 9).	Wide variety of stationary phases are available for selective removal of desired inorganic, organic, and biological analytes; specialty phases exist for drugs of abuse, carbohydrates, catecholamines, metal ions, trace enrichment of water, and many other classes of compounds.
Liquid-Liquid Extraction	Sample is partitioned between two immiscible phases which are chosen to maximize differences in solubility; interference-free analytes are then recovered from one of the two phases (Chapter 7).	Beware of formation of emulsions — break them with heat, addition of salt; values of K_D can be optimized by the use of different solvents or additives (such as buffers for pH adjustment, salts for ionic strength, complexing agents, ion pairing agents, etc.); many published methods; continuous extractions for low K_D values.
Dilution	Sample is diluted with solvent which is compatible with HPLC mobile phase or GC stationary phase; used to avoid column overload, to decrease solvent strength, or for the output signal to be within the linear range of detector.	To avoid excess peak broadening or distortion, dilution solvent should be miscible with, and preferably weaker than, the HPLC mobile phase; "dilute and shoot" is a typical sample prep method for simple liquid samples such as pharmaceutical formulations; for GC, too strong or an incompatible solvent should be avoided to protect coated stationary phases.
Evaporation	Liquid is removed by gentle heating at atmospheric pressure with flowing air or inert gas; vacuum is useful for low volatility liquids.	Do not evaporate too quickly; bumping can lose sample; watch for sample loss on wall of container; do not overheat to dryness; best under inert gas like N_2; rotary evaporator works best; automated systems evaporation systems available.
Distillation	Sample is heated to boiling point of solvent and volatile analytes in the vapor phase are condensed and collected.	Mainly for samples which can be easily volatilized; some samples can decompose if heated too strongly; vacuum distillation can be used for low vapor pressure compounds; steam distillation is rather gentle since maximum temp is 100 °C.
Microdialysis	A semi-permeable membrane is placed between two aqueous liquid phases and analytes transfer from one liquid to the other, based on differential concentration.	Enrichment techniques such as SPE are required to concentrate dialyzates; microdialysis is used for examination of extracellular chemicals in living plant and animal tissue, in fermentation broth; it has been used on-line with microLC columns; dialysis with molecular-weight cutoff membranes can also be used on-line to deproteinate samples prior to HPLC; ultrafiltration and reverse osmosis can be used in a similar manner.

(Continued)

Typical Sample Preparation Methods for Liquids and Suspensions

Methods of Sample Preparation	Principles of Technique	Comments
Lyophilization	Aqueous sample is frozen and water removed by sublimation under vacuum.	Good for non-volatile organics; large sample volume can be handled; possible loss of volatile analytes; good for recovery of thermally unstable analytes, especially biological analytes; inorganics can be concentrated.
Filtration	Liquid is passed through paper or membrane filter or SPE cartridge/disk to remove suspended particulates.	Highly recommended to prevent HPLC backpressure problems and to preserve column life; keeps particulates out of capillary GC columns (Chapter 5)
Centrifugation	Sample is placed in tapered centrifuge tube and spun at high force (thousands to hundreds of thousands times gravity); supernatant liquid is decanted.	Centrifugation is used to remove particulates as an alternative to filtration; ultracentrifugation normally not used for simple particulate removal.
Sedimentation	Sample is allowed to settle when left undisturbed in a sedimentation tank; settling rate dependent on Stoke's radius.	Extremely slow process; manual recovery of different size particulates at different levels, depending on settling rate.
Solid Phase Microextraction (SPME)	see Table 2.1	see Table 2.1
Stir Bar Sorbent Extraction (SBSE)	Analogous to SPME except, the phase ratio is much larger; a magnetic stirring bar usually encased in glass is coated with a polymeric stationary phase. The coated stir bar is placed in a liquid or semi-liquid sample and analytes diffuse and partition/adsorb onto the stationary phase; after removal from the sample, stir bar is dried and like SPME analytes are thermally desorbed in a special desorption unit for GC or washed with an appropriate solvent for HPLC.	SBSE is also an equilibrium method, but because of the larger mass of sorbent on the coated bar, the sample capacity (and hence, sensitivity) of this technique may exceed the SPME technique by a couple of orders of magnitude; SBSE requires a special larger volume thermal desorption apparatus while SPME uses the GC injection port for thermal desorption. The technique is more difficult to automate than SPME.

References

1. *Compendium Method TO-11A: Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC)*, Center for Environmental Research Information, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, **1999**.

2. Schneider, S. *Analysis of DNPH-Derivatized Aldehydes and Ketones Using the Agilent 1220 Infinity LC System with Diode Array Detector*, Agilent Application Note #5991-1545EN, **2013**.

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