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IMPACT OF INDUSTRIAL AND GOVERNMENTAL REGULATORY PRACTICES ON ANALYTICAL CHROMATOGRAPHY

Chromatography is a proven method for separating complex samples into their constituent parts, and it is undoubtedly *the* most important procedure for isolating and purifying chemicals. Using data from the first half of 2003, Ryan estimated that nearly 5% of all chemical research in 2003 would involve chromatography.[1]

In addition, most chromatographic instrumentation is equipped with detectors, making chromatographs true instruments, devices capable of making measurements. Consequently, this monograph will deal not only with the principles of chromatography but also with the practice of quantitative analysis. It is this latter subject that has been greatly influenced by both industry and the federal government because of the need for standards and standardization that go hand-in-hand with governmental regulation. In the modern world, these issues extend to foreign countries as well and have given rise to international organizations and guidances/regulations that need to be recognized by chromatographers worldwide. Since much important information is available on the Internet, all scientists need to be knowledgeable about its retrieval and its impact on their work. In addition, much effort is being made internationally to provide a cooperative and harmonized approach to analysis and analytical method development. Although this book is

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written from the perspective of chromatographers in the United States, the principles are applicable internationally, and scientists would be well advised to recognize that fact and become aware of the developments outside their own countries.

Fortunately, the fundamental principles of chromatography and analytical chemistry in general are the same in academia, industry, and government, of course. Their common objective is to perform laboratory tests and procedures that are based on sound scientific principles. However, some industries operate under more stringent controls than others. For example, the pharmaceutical industry in the United States is regulated by the Food and Drug Administration (FDA), which enforces federal regulations known as the Current Good Manufacturing Practices (CGMPs)*. These regulations were promulgated to ensure the safety and efficacy of drugs by setting forth minimum standards for manufacturing and testing. The GMPs are not prescriptive and, therefore, they have been supplemented by FDA guidance documents that provide more specific details on complying with the regulations. These guidances provide insight for the practice of good chromatography in all venues where analytical chemistry is performed, in the United States and abroad. While it is true that European and Asian counterparts are similarly regulated by their respective agencies, the fundamental analytical principles are the same and are becoming internationally codified.

Because these special regulations and guidances are often omitted from academic courses,[2] this chapter is presented to guide informed readers as they proceed to industrial and governmental employment. It also serves as a general introduction to quantitative analysis practices in chromatography by presenting and summarizing some basics of chromatographic measurement. This chapter examines:

- The organization of analytical chemists in a typical industrial corporation
- The organization and regulatory agencies of the U.S. government and of nongovernmental agencies
- The effect of FDA regulation on the pharmaceutical practices in the laboratory
- Some international guidelines for analytical chemistry in general and analytical chromatography in particular.

*The official title of the FDA regulations includes the word *Current* so the abbreviation should be CGMP. However, some authors use a lowercase *c* and call them the cGMPs, and others shorten the name to just GMP. For simplicity, we will use GMP in most cases.

1.1 LOCUS OF CHROMATOGRAPHY IN CHEMICAL INDUSTRY

Chemical companies and related industries such as pharmaceutical companies and the petroleum industry more than ever need to have laboratories devoted to analysis methods and characterization, including in most cases a section well trained in chromatography. Those that produce and sell chemicals have a laboratory function called quality control (QC) that monitors the quality of incoming raw materials, evaluates in-process intermediates, and tests the purity of final products. Assurance of the quality of manufactured products, referred to as quality assurance (QA) and carried out in conjunction with manufacturing, is a related function. Both functions may be combined, and the laboratory may be called a QC/QA laboratory. This laboratory usually performs both qualitative (identity) analyses and quantitative analyses. The latter are often performed by gas chromatography (GC) or liquid chromatography (LC). Usually, these laboratories are situated close to, or within, the manufacturing site. Typical of many companies hiring B.S. chemists, large pharmaceutical firms hire recent bachelors chemists as analytical chemists into their QC laboratories.[3]

Depending on the size of the company, another laboratory may be responsible for developing the methods for the QC laboratories. This function may be in the Research and Development (R&D) Department. The chromatographers in this laboratory are usually responsible for keeping up with the latest developments in chromatography and searching for and evaluating new improved methods of analysis, as well as developing methods for the QC laboratory. Instrument companies manufacturing chromatographs may also have their own instrumental R&D groups that often provide technical support. Generally, R&D groups are staffed by degree chemists at several levels with some Ph.D.s at the highest levels.

Another analytical need is for a group to perform general analytical services to support the chemical activities of the company (synthesis, pilot plant, product support, etc.). These services most often include chromatography, spectroscopy, and microanalytical (elemental) analysis. Often this is a separate group of scientists and engineers and may include a small group of experts that advises and consults with technicians in the other areas who do their own analytical work. Separate groups may exist to support the sales and marketing department or the patent and law department, for analysis of competitors samples or evaluation of patent infringement, for example.

Within a chemical corporation, these various laboratories are responsible for providing accurate and reliable analytical methodology. The interrelated elements required for this process are shown in Figure 1.1. Each part is important, and some of them will be discussed further in this chapter:

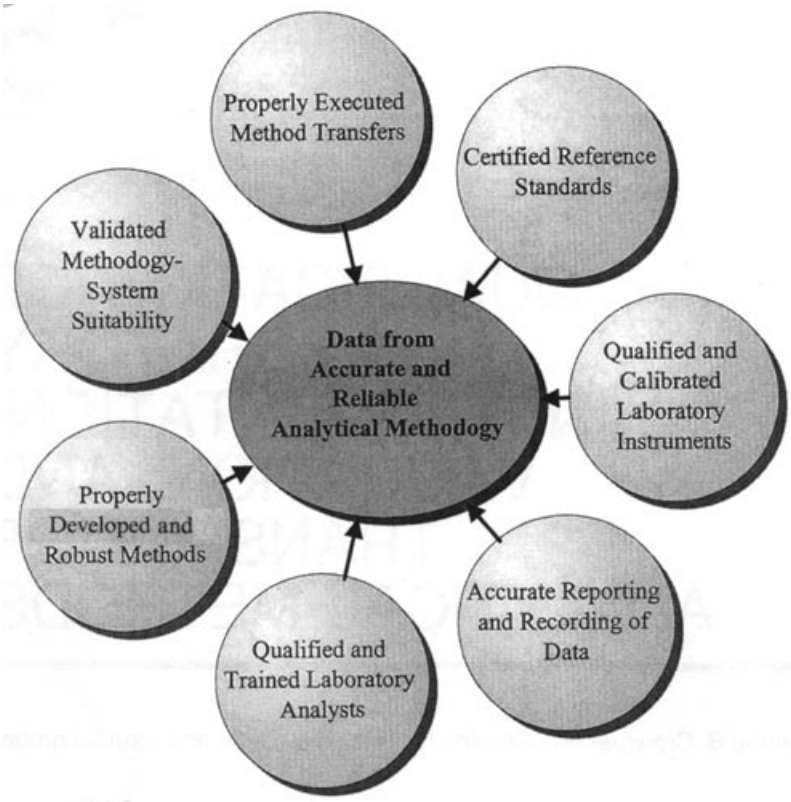

Figure 1.1. Interrelated elements that ensure reliability of data. Reprinted with permission from J. Miller and J. Crowther (eds), *Analytical Chemistry in a GMP Environment*, John Wiley & Sons. Copyright 2000; this material is used by permission of John Wiley & Sons, Inc.

standards, instrument qualification, and method development and validation.

In general, government laboratories are organized similarly. Some of them are of particular interest to analysts because of the functions they perform, including the regulation of industrial practice.

1.2 GOVERNMENTAL ORGANIZATIONS

Table 1.1 lists some U.S. government laboratories and agencies that are of interest to chromatographers. Those that are part of a governmental department are listed by department in order to show the governmental organization. The ones of greatest interest to chromatographers, and the ones discussed in greatest detail in this chapter, are the National Institute of Standards and Technology (NIST), the Food and Drug Administration (FDA),

Table 1.1 U.S. Government Laboratories and Scientific Agencies and Departments

Departments
Agriculture (USDA). Over 100 research labs nationwide.
Commerce. Includes the National Institute of Standards and Technology (NIST), formerly the National Bureau of Standards (NBS).
Energy (DOE). Sixteen laboratories including the famous ones at Argonne, Brookhaven, Los Alamos, and Oak Ridge.
Health and Human Services (HHS). Includes the Centers for Disease Control and Prevention (CDC) and its division, the National Institute for Occupational Safety and Health (NIOSH); the Food and Drug Administration (FDA); and the National Institutes of Health (NIH).
Justice. Includes the Drug Enforcement Agency (DEA) and the Federal Bureau of Investigation (FBI).
Labor. Includes the Occupational Safety and Health Administration (OSHA).
Interior. Includes the U.S. Geological Survey (USGS).
Treasury. Includes the Bureau of Alcohol, Tobacco and Firearms (ATF).
Other
Environmental Protection Agency (EPA)
National Science Foundation (NSF)

and the Environmental Protection Agency (EPA) because they are most involved in standards, standardization, method development, and federal regulation. The U.S. government web site (*www.firstgov.gov*) can be used to locate additional information on government agencies and federal regulations.

National Institute of Standards and Technology (NIST)

The mission of NIST (formerly the National Bureau of Standards, NBS) is "to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve quality of life."[4] It was founded in 1901, making it the oldest physical science research laboratory of the federal government.[5] Unlike the FDA and the EPA, it is not a regulatory agency and does not establish or enforce mandatory standards; rather, NIST develops measurement methods, instrumentation, and measurement standards for government and industry.[6]

The main NIST laboratory is outside Washington, D.C., in Gaithersburg, Maryland, and the second one is in Boulder, Colorado. One of the eight laboratory divisions, the Chemical Science and Technology Laboratory

(CSTL), includes an Analytical Chemistry section that is divided into five groups. One of them is the Organic Analytical Methods group where separation methods, including most of chromatography, is located. CSTL performs services like those described above for R&D departments; it "conducts research in measurement science and develops the chemical, biochemical, and chemical engineering measurements, data, models, and reference standards" for the United States.[4]

Reference standards are particularly important in analytical chemistry, and a later section of this chapter is devoted to that topic. The Analytical Chemistry section of the CSTL is responsible for 850 of the 1350 NIST standards, called standard reference materials or SRMs.[6] On the occasion of its attaining the age of 100, the NIST published a booklet chronicling the first century of SRMs.[7] Some chromatographic examples of SRMs are:

869a for LC selectivity
870 for LC performance
877 for LC chiral selectivity
1543 for gas chromatography/mass spectrometry (GC/MS) performance.

As an illustration of the nature of SRMs, 869a is a mixture of three polycyclic aromatic hydrocarbons (PAHs) in acetonitrile, useful for characterizing LC column selectivity for the separation of PAHs.

The NIST also provides a wide range of publications and databases. Called the NIST Virtual Library, they can be accessed online at *nvl.nist.gov*.

Worldwide coordination and cooperation between the individual standardization agencies is also a task of NIST. Globally recognized measurements and standards are being developed through the efforts of many national metrological institutes worldwide, through the signing of a Mutual Recognition Arrangement (MRA) whereby 50 national standards laboratories have agreed to participate in formal interlaboratory comparisons.[8] The responsibility for this effort in the United States is carried mainly by the Analytical Chemistry section of the NIST.

Food and Drug Administration (FDA)

The FDA is a regulatory agency formed as a result of the government's Food, Drug and Cosmetic Act (FD&C act) in 1938. Simply stated, its mission is "to promote and protect the public health by helping safe and effective products reach the market in a timely way, and monitoring products for continued safety after they are in use."[9] In addition to food and drugs, the FDA regulates cosmetics, medical devices (such as pacemakers), biologics (such as

vaccines), animal feed and drugs, and radiation-emitting products (such as cell phones). Its web site[9] contains a wealth of information, some of which is indicated in the flowchart[10] in Figure 1.2. The focus in this chapter will be on drugs.

There are about 10,300 FDA-approved drugs in the United States today,[9] and the division of the FDA responsible for most of them is the Center for Drug Evaluation and Research (CDER). The Center for Biologics Evaluation and Research (CBER) is responsible for biologicals, and the Center for Veterinary Medicine (CVM) regulates veterinary drug products. The CDER reviews applications for new drugs (NDAs) and generic products (ANDAs) and oversees the quality and manufacturing of drugs by participating in on-site inspections with the office of regulatory affairs (ORA). The regulations it enforces are federal laws called Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP), or alternatively, Current Good Manufacturing Practice (CGMP) as noted earlier.* One might think that chromatographers would be most concerned with GLPs, but that is not the case. It is primarily the GMPs that provide the regulations applied by laboratories to give assurance that the *manufactured* products meet specifications. GLPs mostly concern the conduct of nonclinical laboratory (toxicology) studies, while GCPs address Good Clinical Practices. All of these regulations are sometimes lumped together and referred to as GXPs when not referring to a specific regulation.

The necessity to conform to the applicable GXPs has had major effects on the operation of analytical laboratories in the pharmaceutical industry; many of these basic business principles outlined in the GMPs have been adopted by others in the wider analytical community. A major requirement regarding analytical methods is that they must be validated. Method validation is the process of acquiring data and documentation to prove that a specific method will produce reliable data with a high degree of assurance and is therefore acceptable for its intended purpose. The measures for evaluating a quantitative method, such as a high-pressure liquid chromatographic (HPLC) analysis, include accuracy, precision, specificity, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), robustness, and sensitivity [added later by an International Conference on Harmonisation† (ICH) guideline]. An equally important requirement is that instrumentation used in the testing method and during validation activities must also meet stringent controls referred to as instrument qualifications. As a matter of clarification, in

*See page 2.

†Spelling harmonisation with an *s* is the British version. In this text, when a European group or agency is being referenced, the British spelling will be used.

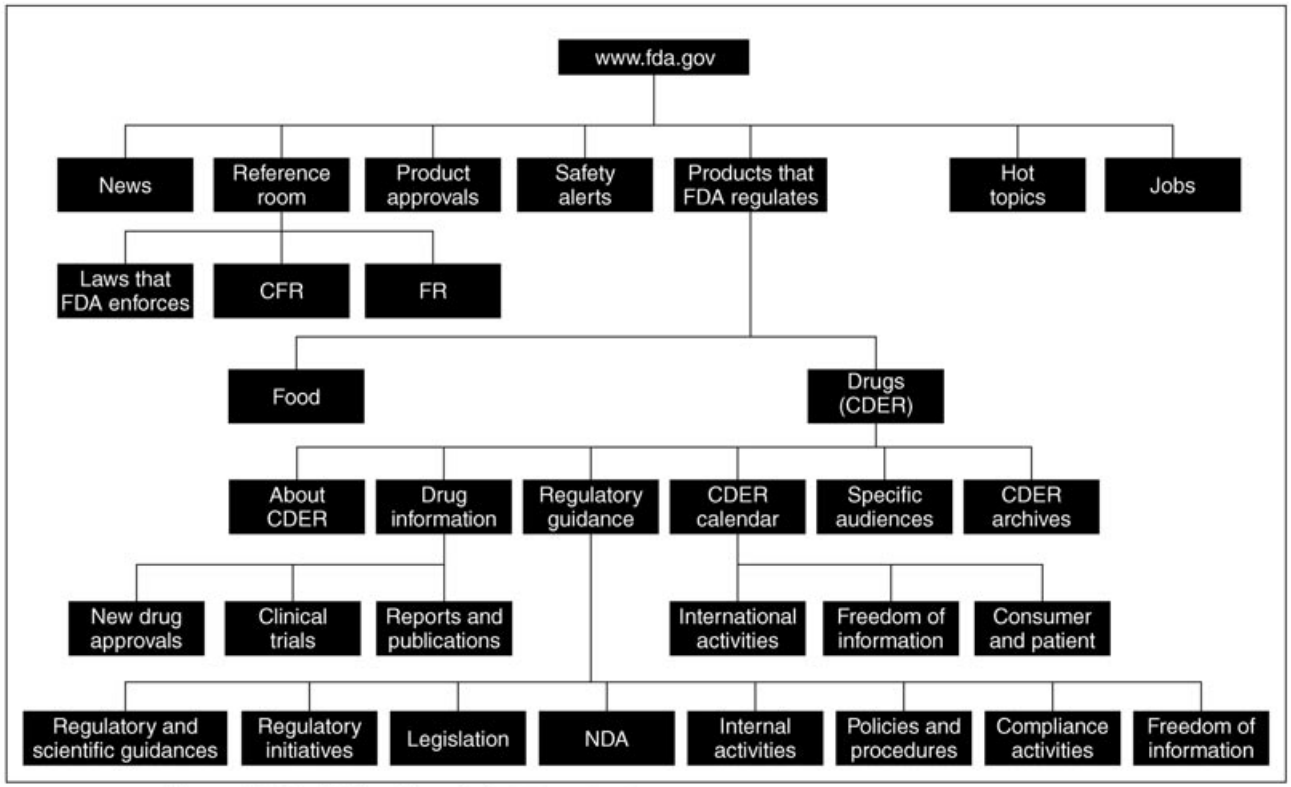

Figure 1.2. Partial flowchart of the FDA web site. Reprinted with permission from *LC-GC Europe*, Vol. 16(1), January 2003, p. 40. *LC-GC Europe* is a copyrighted publication of Advanstar Communications, Inc. All rights reserved.

general, instruments are qualified and processes (methods) are validated. Further details on these subjects are deferred until later in this chapter.

There are other key compliance issues in addition to method validation and instrument qualification including[11]:

- Management systems
- Operating procedures
- Personnel training
- Data accountability
- Facility adequacy and compliance
- Certification documentation

Surely this list represents requirements that one would expect to address when attempting to improve one's laboratory practices. Although detailed discussion of all of these topics is beyond the scope of this monograph, some of the most important issues are addressed; additional information can be found in the published literature.[11, 12]

The GMPs are published by the National Archives and Records Administration and the Government Printing Office (GPO) in the Code of Federal Regulations (CFR), which is a codification of the general and permanent rules published by the executive departments and agencies of the federal government. It can be accessed online from the FDA web site or directly at *www.gpoaccess.gov / cfr / index.html*. The CFR is divided into 50 titles, which represent broad areas subject to federal regulation; the GMPs are in Title 21, as listed in Table 1.2. Before final publication and becoming law, new proposed regulations are first published for review in the *Federal Register* (FR), also accessible from the FDA site, as well as directly at

Table 1.2 Sectionss of CFR Title 21. GXPs

Part 11	Electronic records; electronic signatures
Parts 50, 54, 56	GCP for clinical laboratories
Part 58	GLP for nonclinical laboratories
Part 110	CGMP in manufacturing, packing, or holding human food
Part 210	CGMP in manufacturing, processing, packing, or holding of drugs
Part 211	CGMP for finished pharmaceuticals (GMPs)
Part 600	Biological products
Part 610	General biological products standards
Part 820	Quality system regulation

www.gpoaccess.gov/fr/index.html. Free online access to these federal regulations is a service only recently made available, and one that should be widely exploited.

Surprisingly, the FDA does not prescribe methods. In the pharmaceutical industry in the United States, the methods most often used are those published by the U.S. Pharmacopeial Convention (USP/NF), which is not a federal agency or publication. The USP publishes methods, many of which have been approved by the FDA, but the FDA does not *submit* methods to USP. Companies are free to use their own methods as long as they are as good as, or better than, the USP/NF methods. Consequently, method development is done by companies and universities and is based on the regulations published by the FDA. The FDA (in particular, the CDER) and the USP/NF work very closely together; the FDA reviews and comments on USP information and standards. To be in compliance with FDA regulations, a pharmaceutical laboratory has to provide data and documentation to show that its methods meet the requirements published in the USP/NF.

The GMP regulations in Title 21 of the CFR are general, and they are not specific enough to be enforced without further elaboration. Discussion of, and comments about, regulations are often presented online at the FDA site, and compliance issues are also addressed in the USP publication, *Pharmacopeial Forum* (PF). The FDA publishes *CGMP Notes* quarterly. These notes are intended to clarify issues and answer questions related to interpretation of the GMP regulations. They are not regulations and are primarily for internal FDA use; they can be accessed at *www.fda.gov/cder/dmpq/cgmpnotes.htm*.

The FDA publishes guidelines or guidance documents in an attempt to clarify the intent of the regulations it intends to enforce. In 1997, in an attempt to be more specific about the intent and meaning of the term *guidance*, the FDA published in the FR a notice on guidance documents.[10] In effect, it created a new category of GXPs, Good Guidance Practices (GGPs), setting forth its policies and procedures for developing, issuing, and using guidance documents. The notice states; "Guidance documents do not themselves establish legally enforceable rights or responsibilities and are not legally binding on the public or the agency. Rather, they explain how the agency believes the statutes and regulations apply to certain regulated activities."[13] The majority of future guidance documents will be labeled either (1) compliance guidance, (2) guidance for industry, or (3) guidance for FDA reviewers and staff. Type 2 documents are of primary interest to chromatographers in the laboratory and are the ones receiving the most attention in this chapter.

An example of guidance documents on method validation is *Validation of Chromatographic Methods*[14] issued in 1994 (typical of guidance documents

issued before the 1997 statement and originally published in the FR). Other examples of draft guidance documents include *Analytical Procedures for Methods Validation*[15] issued August, 2000, another concerning out-of-specification (OOS) results,[16] and one on residual solvents.[17] They can be downloaded from the FDA CDER site at *www.fda.gov/cder/guidance/index.htm* and also from CBER at *www.fda.gov/cber/publications.htm*. Other FDA information of interest to pharmaceutical chromatographers can be found in reference 10.

Furthermore, since many American pharmaceutical companies market their drugs outside the United States, they then need to meet the requirements of foreign regulatory agencies as well as those of the FDA. In fact, a worldwide effort to achieve common requirements and international cooperation has been ongoing since the early 1990s and is called the International Conference on Harmonisation (ICH). A later section in this chapter is devoted to the USP/NF and international pharmacopoeias.

Environmental Protection Agency (EPA)

The EPA has been working for over 30 years to protect human health and to safeguard the natural environment (air, water, and land) in the United States. It develops and enforces environmental regulations through 10 regional offices and 17 laboratories. The regulations are codified in Title 40 of the CFR, which can be accessed online from the EPA web site: *www.epa.gov*.

The EPA Office of Research and Development (ORD) has publications in the following areas: general, air, EMPACT, multimedia, pollution prevention, risk, risk assessment guidelines, STAR grant research, waste, and water.[18] Laboratory methods of interest to chromatographers can be found in the eight Laboratory Analytical Chemistry Methods Manuals, covering the topics listed in Table 1.3. They were originally published by the former

Table 1.3 EPA Laboratory Analytical Methods Manuals

1. Methods for the Determination of Organic Compounds in Drinking Water; EPA-600/4-88/039
2. Supplement I of Organics Manual; EPA-600/4-90/020
3. Supplement II of Organics Manual; EPA-600/R-92/129
4. Supplement III of Organics Manual; EPA-6000/R-95/131
5. Methods for the Determination of Inorganic Substances in Environmental Samples; EPA-600/R-93/100
6. Methods for the Determination of Metals in Environmental Samples; EPA-600/4-91/010
7. Supplement I of Metals Manual; EPA-600/R-94/111
8. Methods for the Determination of Chemical Substances in Marine & Estuarine Environmental Samples; EPA-600/R-92/121

Environmental Monitoring Systems Laboratory in Cincinnati between 1988 and 1995. The scope of this project can be seen from the fact that the 1988 *Manual on Methods for the Determination of Organic Compounds in Drinking Water* contains 13 methods cross-indexed to over 200 analytes. The individual methods are listed at the web site *www.epa.gov / nerlcwww / methmans.html* and can be purchased from the National Technical Information Service (NTIS).[19]

Other Organizations

Two other agencies listed in Table 1.1 have issued standard chromatographic methods of analysis. They are the National Institute of Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA). From their names one would expect them to be in the same department, but NIOSH is a subsection of Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services (HHS), and OSHA is in the Department of Labor. Like EPA they have published many methods, some covering the same chemicals. Both agencies methods are available in print and online.[20, 21]

1.3 NONGOVERNMENTAL AGENCIES

The most relevant nongovernmental agencies and societies are listed in Table 1.4 along with their web sites and some of the relevant activities in which they engage. The latter include activities such as specification of standards and standardization (S), development and recommendation of analysis methods (M), recommended regulations (R), recommendations of nomenclature and definitions (N), and international activities promoting harmonization and cooperation (I). Many of them also publish reports and journals; some are active only in the United States while others (often identified by their names) are international. Four have been chosen for extensive commentary in this section: Association of Analytical Communities International (AOAC), American Society for Testing and Materials (ASTM) International, International Organization for Standardization (ISO), and International Union of Pure and Applied Chemistry (IUPAC). Before discussing them, a few comments will be made about a few of the others, but many of the 25 agencies listed in Table 1.4 cannot be included in this brief section. Internet URLs (universal resource locators) are given for the purpose of obtaining additional information about them.

The American Chemical Society (ACS) is probably well known to all readers. Its activities, in the context of this discussion, are exemplified by its publication of specifications for reagent chemicals.[22] They are the specifications for the quality grade of over 400 chemicals referred to as ACS reagents.

Table 1.4. Nongovernmental Agencies and Societies

Name	Web Site	Activity[a]	Home
1. American Chemical Society (ACS)	*www.acs.org*	N, S	Washington, D.C.
2. American National Standards Institute (ANSI)	*www.ansi.org*	I, S	Washington, D.C.
3. American Public Health Association (APHA)	*www.apha.org*	M	Washington, D.C.
4. American Water Works Association (AWWA)	*www.awwa.org*	M	Denver, CO
5. Analytical & Life Science Systems Association (ALSSA), formerly Analytical Instrument Assoc. (AIA)	*www.alssa.org*	S	Alexandria, VA
6. Association of Analytical Communities (AOAC) International	*www.aoac.org*	I, M, R, S	Gaithersburg, MD
7. American Society for Quality (ASQ, formerly ASQC)	*www.asq.org*	I, S	Milwaukee, WS
8. ASTM International	*www.astm.org*	I, M, N, S	W. Conshohocken, PA
9. Cooperation on International Traceability in Analytical Chemistry (CITAC)	*www.citac.cc*	I, S	Geel, Belgium
10. Collaborative Electronic Notebook Systems Association (CENSA)	*www.censa.org*	I	Woburn, MA
11. Eurachem	*www.eurachem.ul.pt*	I, M, S	Portugal
12. Instrumentation, Systems, and Automation Society (ISA)	*www.isa.org*	I, S	Research Triangle Park, NC
13. International Atomic Energy Commission	*www.iaea.org*	I, M, S	Vienna
14. International Conference on Harmonisation (ICH)	*www.ich.org*	I, M, N, R, S	Geneva
15. International Electrotechnical Commission (IEC)	*www.iec.ch*	I	Geneva
16. International Laboratory Accreditation Cooperation (ILAC)	*www.ilac.org*	A, I, S	Netherlands
17. International Organization for Standardization (ISO)	*www.iso.ch*	I, S	Geneva
18. International Society for Pharmaceutical Engineering	*www.pharmaceuticalonline.com*	I	Tampa, FL
19. International Union of Pure and Applied Chemistry (IUPAC)	*www.iupac.org*	I, N, S	Research Triangle Park, NC
20. Institute for Reference Materials and Measurements, EC (IRMM)	*www.irmm.jrc.be*	I, S	Geel, Belgium
21. National Conference of Standards Laboratories (NCSL) International	*www.ncsli.org*	I, N, S	Boulder, CO
22. Organisation for Economic Cooperation and Development (OECD)	*www.oecd.org*	I, N, R, S	Paris
23. Product Quality Research Institute (PQRI)	*www.pqri.org*	I, R	Arlington, VA
24. U.S. Pharmacopeial Convention (USP)	*www.usp.org*	M, N, S	Rockville, MD
25. Water Environment Federation (WEF)	*www.wef.org*	M	Alexandria, VA

Activities: I, international harmonization and cooperation; M, publication and/or development of methods; N, nomenclature and definitions; R, regulations/regulatory; S, standards, standardization, and protocols.

This book also contains useful definitions and procedures for analytical chemistry, including those on chromatography. An online demonstration of the web edition can be accessed at *http://pubs.acs.org/reagents/index.html*. Updates to the ninth edition are also posted there.

Another useful monograph gives standard methods of analysis for water and wastewater.[23] It has been published in a collaborative effort of the American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF) and contains over 350 separate test methods. Furthermore, the EPA has just given regulatory approval to this latest edition, making it an official manual for EPA methods.

A joint venture headed by the Analytical Instrument Association (AIA, now renamed ALSSA, Analytical and Life Science Systems Association) has produced protocols for chromatographic data interchange. Called ANDI protocols (for analytical data interchange), they are intended to increase laboratory efficiency and productivity by facilitating the integration and use of data from multiple vendors' instruments.[24] Nine chromatographic instrument companies are currently participating, and ASTM has adopted these protocols.[25] Further information and references can be found in references 24 and 25.

Association of Analytical Communities International (AOAC)

The AOAC started out in 1884 as the Association of Official Agricultural Chemists under the U.S. Department of Agriculture (USDA), then became the Association of Official Analytical Chemists, and since 1991 is the Association of Analytical Communities; the abbreviation for all of these names has remained AOAC, but the breadth of the organization has increased. It has always been one of the leading organizations producing standards for analytical chemists. Its book of "official methods" is in its 17th edition[26] and contains 2800 tested analytical methods. It is the single most comprehensive collection of validated analytical methods available anywhere.

The AOAC International has written three method validation programs and administers many contracts with other agencies and organizations, such as the FDA and the USDA. Its activities are no longer limited to regulatory functions as was implied when the term "official" was part of its name, and increasing emphasis is being placed on international collaboration and cooperation. Further details are available at its web site (see Table 1.4).

ASTM International

The ASTM's original full name, American Society for Testing and Materials, reveals that it was originally established to develop and publish standard test

Table 1.5 Section Contents of the Annual Book of ASTM Standards

No.	Topic
1	Iron and Steel Products
2	Nonferrous Metal Products
3	Metals Test Methods and Analytical Procedures
4	Construction
5	Petroleum Products, Lubricants, and Fossil Fuels
6	Paints, Related Coatings, and Aromatics
7	Textiles
8	Plastics
9	Rubber
10	Electrical Insulation and Electronics
11	Water and Environmental Technology
12	Nuclear, Solar, and Geothermal Energy
13	Medical Devices and Services
14	General Methods and Instrumentation
15	General Products, Chemical Specialties, and End Use Products

methods primarily for America. Now, it is significantly engaged in worldwide issues, as appropriately reflected in its change of name to ASTM International. ASTM is not a regulatory agency, and its methods are voluntary and are arrived at by consensus among groups of interested scientists. Over 30,000 individuals from 100 nations are members of ASTM International, evidence that it is no longer restricted to America. The methods have been published annually for many years, and currently cover over 11,000 standards in 15 sections contained in more than 70 volumes.[27] Table 1.5 lists the 15 sections, most of which are related to manufactured products. The contents can be searched online by title and by subject at the ASTM web site, but the text is only available for a fee, in print or online.

Typical methods of interest to chromatographers include D6420-99 Standard Test Method for Determination of Gaseous Organic Compounds by Direct Interface Gas Chromatography–Mass Spectrometry (Vol. 11.03) and D6156-97 Standard Practice for Use of Reversed-Phase High Performance Liquid Chromatographic Systems (Vol. 11.02).

The ASTM International has also published 6 sets of data (designated DS), 44 manuals (designated MNL), 1434 special technical publications (designated STP), and 5 journals. Some examples of STPs are:

STP 577 Calculation of Physical Properties of Petroleum Products from Gas Chromatographic Analysis

STP 1161 Leak Detection in Underground Storage Tanks

STP 1223 Standardization and Harmonization Terminology: Theory and Practice

Most of its work is done by technical committees that have broad representation to assure wide consensus. While most are on specific materials, the E-committees cover miscellaneous subjects, and E-19 is the one on chromatography.

International Organization for Standardization (ISO)

The ISO is a nongovernmental, international organization established in 1947. The name, ISO is obviously not an acronym for its name. In English, the prefix *iso* is used to denote "same"; in fact, *iso* is derived from the Greek *isos*, meaning "equal." Being a truly international organization from the start, ISO chose as its name an acronym that is internationally recognized as denoting same or equal or standard, regardless of the language of the user. The ISO's work results in international agreements, over 13,500 of which have been published as international standards.

Standards are classified among 40 different fields. Number 19 is Testing and number 71 is Chemical Technology. Analytical Chemistry is number 71.040, and subsection 50 (71.040.50) includes chromatographic analyses. A key word search of the online catalog turned up 80 methods using chromatography. Some examples are: ISO 7609:1985 Essential Oils—Analysis by Gas Chromatography on Capillary Columns; ISO 14718:1998 Animal Feeding Stuffs—Determination of Aflatoxin B1 Content of Mixed Feeding Stuffs; a Method Using High-Performance Liquid Chromatography.

A general standard of particular interest to chromatographers is ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories, published in 1999.[28] The 30-page document is not available online, but it can be ordered from the web site. Somewhat like the GMPs of the FDA, it deals with general areas such as terms and definitions, management requirements, and technical requirements. It addresses issues including quality systems, personnel, internal audits, method validation, sampling, standards, equipment, and data handling.

Finally, ISO 9000 is a family of standards dealing with quality management systems. They are generic standards, meaning that they can apply to any organization that wishes to enhance customer satisfaction by meeting customer needs and regulatory requirements. Most chromatography manufacturers and suppliers have conformed to these standards and have issued ISO 9000 certificates. Dealing with these companies should be better as a result. It should be noted, however, that ISO does not carry out the certification and does not issue certificates.

The American National Standards Institute (ANSI, see Table 1.4) is the official U.S. representative to ISO. It is a private, nonprofit organization that administers and coordinates the U.S. voluntary standardization and conformity assessment system.[29] It has done so for over 80 years and currently has approximately 1000 members, representing industrial companies, organizations, government agencies, and other institutions. It is located in Washington, D.C. More information is readily available at its web site (see Table 1.4). It should be noted that ASTM International is also a cooperating agency with ISO and has sponsored or co-sponsored many of its committees.

International Union of Pure and Applied Chemistry (IUPAC)

The IUPAC is the granddaddy of international chemical bodies. Since its beginning in 1919 it has served worldwide as the primary agency fostering harmonization among chemical groups, industrial and academic. It has long been recognized as the authority on chemical nomenclature and terminology, atomic weights, and standardized methods for measurements. Of its eight divisions, analytical chemistry is division 5.

The IUPAC publishes three journals including *Pure and Applied Chemistry* and has an online newsletter, *Chemical Education International* (see Table 1.4 for web site). Its published books include a series on solubility data,[30] a periodic handbook,[31] and a compendium on analytical nomenclature.[32] The latter can be accessed online; Chapter 9 is on Separations. The IUPAC recommendations on chromatographic nomenclature were originally published in 1993[33] and resolved many conflicting symbols and terms. Its recommendations will be used throughout this book and further discussion is presented in Chapter 2. Some current projects of IUPAC include harmonization of international quality assurance schemes for analytical laboratories and studying the definitions of asymmetrical chromatographic peaks.[34]

1.4 STANDARDS, CALIBRATION, AND NIST

One of the important steps in any analytical method involves calibration with appropriate standards. Prior discussion in this chapter has included some information on this topic and has given an indication of the number of organizations interested in it. This section will attempt to summarize the main aspects of the calibration process and collect in one place the contributions of the various organizations.

The term *standardization* can be used in a number of different situations. For example, the attempts by various organizations to agree on a specification, a method, or a definition are all examples of the process of standardization. Alternatively, an example of standardization in the laboratory is the

process of comparing the strength of a solution against a standard. The preparation of such a "standard" may require its purchase from a supplier of standards, or it may be the process of comparing a newly prepared solution against a certified standard, thus producing a "secondary standard" or "working standard." Let us examine the laboratory standardization process as it is commonly practiced by chromatographers.

First is the process of agreeing on a method to be used for a particular analysis. Earlier we discussed those agencies that are concerned with the process of arriving at approved, standard methods. There are more than 400 organizations in the United States alone dedicated to this purpose.[6] Their standards can be mandatory or voluntary. Most stringent are the mandatory standards that are enforced on a regulated industry by the government. If one works for a pharmaceutical company, for example, the method to be used will be the one approved by the FDA. Once approved by the FDA, pharmaceutical companies, and other interested parties, usually submit their methods to the USP/NF for publication, although this may not occur for several years after FDA approval and they are not obligated to do so. Some standards are arrived at by consensus from among the constituents who will use it, usually on a voluntary basis. ANSI is closest to being the centralizing voice for standards development in the United States.[35] As stated earlier, ANSI is also the U.S. representative to ISO. Others we have discussed are so indicated in Tables 1.1 and 1.4.

The standards just discussed can be referred to as standard test methods, but there is also another type: standard recommended practices.[35] The latter are generalized procedures, not specific instructions. They are recommended practices for various types of analysis and relevant test methods.

Next is the process of standardizing the instrumentation in one's own lab; this process is called instrument qualification and it will be discussed in a later section.

Finally, there is the process of standardizing the method of analysis, the process we called method validation. Extensive discussion of this procedure is given later in this chapter. Two short monographs prepared by ASTM International provide further details about standardization.[6, 35]

The standards used in standardization can be obtained from a number of sources. The official governmental source, NIST, calls its standards SRMs, Standard Reference Materials, as noted earlier. A recent publication of a conversation with the current chief of the Analytical Chemistry Division of NIST contains interesting material about SRMs.[5]

Other agencies (including ISO) use other names such as Reference Materials (RM) and Certified Reference Materials (CRM).[36] The official source for FDA methods is the USP whose list of standards are called Reference Standards (RS) now available online as well as the print version of USP/NF,

General Chapter 11. Many analytical chemistry texts simply refer to primary standards and probably mean NIST standards. But, obviously, there are many different names used to identify suitable standards, and the one used usually depends on the context in which it is being used. Several chromatography supply houses sell standards for USP, EPA, and other standard methods;[37] some are traceable to NIST.

In most cases standards are chemicals, and as such they must be of known purity and stable. Some may require oven drying prior to use. Some may have retest dates beyond which recertification is required. The general practice in the pharmaceutical industry is that expiration dates are final, but expiration dates are not usually attached to standards. See reference 11 for further information and reference 38 for an international guide for laboratories and accreditation bodies.

For many new methods, no standards are available, of course. In that case, attempts are made to purify available chemicals as much as possible; they are then analyzed by more than one method to ensure proof of purity. If the identity of a chromatographic peak is totally unknown, as is often the case with small impurity peaks, obviously no standard can be prepared until an identification can be made and a method of synthesis is worked out. Much effort is required in this case, and often analyses must be performed without qualified standards. Quantitative analysis in such cases is often performed by the method called area normalization (see Chapter 9) that may be very inaccurate.

1.5 USP AND OTHER PHARMACOPEIAS

Earlier discussion of the FDA's regulation of the pharmaceutical industry discussed the role played by the USP/NF. This section will provide more information about the USP and its activities toward harmonization with pharmacopeias of other nations. The symbol USP can be used in two different ways. It can refer to the organization, the U.S. Pharmacopeial Convention or to its major publication,[39] the USP/NF. Usually there is no confusion if the acronym is used, but if the usage is not clear, the full name will be written out.

The U.S. Pharmacopeial Convention (see Table 1.4) was formed in 1820 and began publication of its pharmacopoeia. The latter served as a guide to drugs for physicians and pharmacists but had no legal status until the passage of the first Pure Food and Drug Act in 1906. It was combined with a similar publication, the *National Formulary* (NF) of the American Pharmaceutical Association in 1974 when both were named as the official U.S. compendia

Table 1.6 Contents of the USP/NF

USP	NF
Introduction	Preface
General Notices (GN)	Admissions (submissions since last edition)
Official Monographs	General Notices (GN)
General Chapters[a]	Combined Index
11 Reference Standards	
201 TLC Identification Test	
467 Organic Volatile Impurities	
621 Chromatography	
726 Electrophoresis	
727 Capillary Electrophoresis	
1078 GMPs	
1196 Pharmaceutical Harmonization (Information from the Pharmaceutical Discussion Group)	
1225 Validation of Compendial Methods	
1251 Weighing on an Analytical Balance	
Reagents	
Reference Tables	
Nutritional Supplements	

[a]Chapters less than 1000 are general requirements for tests and assays; those above 1000 are only informational.

under the Federal Food, Drug and Cosmetic Act.[40] The USP was designated to cover drug substances and dosage forms, and the NF, pharmaceutical ingredients. Both have been published together in one volume for many years and the latest version (reference 39) for the year 2004 is USP 27 and NF 22. It is available in print as a single volume, or on CD, or online. Currently, it is revised and reprinted every year. Two supplements are published each year between the annual revisions to keep it up-to-date.

Table 1.6 shows partial contents of the USP/NF. The General Notices (GN) contain basic information about the volume that should be read, especially by chemists in the pharmaceutical industry. The Official Monographs make up the largest part of the USP; it is an alphabetical listing of USP drugs with USP monographs. The general chapters section includes assays, tests, and determinations including Chapter 621 on chromatography.

The contents of the chromatography section (about a dozen pages) are listed in Table 1.7. System suitability will be defined in the next section and the terms and symbols will be discussed in Chapter 2.

Another publication of USP is the *Pharmacopeial Forum* (PF), a bimonthly journal that previews upcoming changes in the USP. It provides the opportu-

Table 1.7 List of Contents of Chromatography Chapter 621 of USP

Introduction
Paper Chromatography (PC)
Descending Chromatography
Ascending Chromatography
Thin-Layer Chromatography (TLC)
Continuous Development TLC
Column Chromatography (low pressure)
Column Adsorption Chromatography
Column Partition Chromatography
Gas Chromatography
High-Pressure Liquid Chromatography (HPLC)
Size Exclusion Chromatography (SEC)
Interpretation of Chromatograms (N, R_S, α)
System Suitability
Glossary of Symbols
Chromatographic Reagents
Packings
Phases
Supports

nity to comment on proposed changes before they become official. Another publication, *Chromatographic Reagents*, is a reference to brand names of column reagents listed in USP/NF and PF.

Other countries have their own pharmacopoeias, of course, and as international trade has developed, it has become necessary to coordinate and "harmonize" these various publications. The other major pharmacopoeias are the European (EP), British (BP), and the Japanese (JP).[11, 40] Three of the four (BP was not included) have formed a new organization, the International Conference on Harmonisation (ICH) in the early 1990s. The position of the FDA regarding this effort was reported in the *Federal Register* in 1995,[41] thereby beginning a commitment to participate in, and cooperate with, the ICH.

International Conference on Harmonisation Guidelines

The topics addressed by the ICH are (1) quality, Q; (2) safety, S; (3) efficacy, E; and (4) other multidisciplinary, M. Some of the quality topics of interest are listed in Table 1.8, which includes the *Federal Register* citation where they were published by the FDA. Proposed new guidelines such as these go through five steps: step 2 opens the proposal for comments and step 4 is the

Table 1.8 Selected ICH Quality Topics

	Reference
Q2: Analytical Validation	
Q2A: Text on Validation of Analytical Procedures	*Fed. Reg.* **1995**, *60*, 11260
Q2B: Methodology	*Fed. Reg.* **1997**, *62*, 27463–27467
Q3: Impurities	
Q3A(R): Impurities in New Drug Substances	*Fed. Reg.* **2003**, *68*, 6924–6925
Q3B(R): Impurities in New Drug Products	*Fed. Reg.* **2003**, *68*, 64628–64629
Q3C: Impurities: Residual Solvents	*Fed. Reg.* **1997**, *62*, 67377
Q4: Pharmacopoeias	
Q6: Specifications	
Q6A: Chemical Substances	*Fed. Reg.* **2000**, *65*, 83041–83063
Q7: GMP	
Q7A: GMP for Active Pharmaceutical Ingredients	*Fed. Reg.* **2001**, *66*, 49028–49029

final draft. When step 2 or 4 has been reached, the FDA publishes the newly proposed guidances in the FR. Step 4 guidance documents are available for use on the date they are published in the FR, which is ICH step 5. It is easy to follow this progression because the FR is available online, as are most FDA and ICH documents.

An example of the progression of a document through the ICH approval process is the ICH Harmonised Tripartite Guideline: "Text on Validation of Quantitative Procedures, Q2A," which was published (step 2) by the FDA in the FR on March 1, 1994 (58 FR 9750). Comments were accepted until May 16 of that year, and then ICH published it on October 27, 1994 (available from the ICH web site). The FDA published this document subsequently in the FR on March 1, 1995, Vol. 60, pages 11259 to 11262 (available from the *gpoaccess* web site). This final FDA document is also listed in the guidance documents section of the FDA web site at *www.fda.gov/cder/guidance/ichq2a.pdf*. It is this guideline that contains the ICH glossary on validation, including the definitions of basic terms such as *precision* and *accuracy*. That part of the document can be found in Appendix A of this book.

As of April 2000, the FDA has changed its policies somewhat; it now publishes in the FR only a notice that an ICH guidance is in step 5. The complete text of the actual guidance document is made available by the FDA, in print and at the web sites mentioned earlier.

1.6 INTERNATIONAL GUIDELINES FOR ANALYTICAL LABORATORIES

Many of the citations given thus far in this chapter covering the concept of good laboratory practice are rather general and have had the FDA GMPs as

the central focus. The issues raised are important for analytical chromatographers. More important, however, are operating guidelines for chromatographic practice that have been written as a result of the general recommendations. The GMP regulations are not very specific. It is the guidances that provide the details needed for the laboratory. The GMPs may say that analysts need to be trained, but what we need to know is how, when, and by whom. Another example is the validation document discussed above, which includes the definition of limit of detection but not an equation for its calculation. The calculations can be found in ICH Guideline document Q2B, and in this section we will consider it and other sources of specific recommended practices for chromatographers. However, at this time there is no single internationally agreed upon set of guidelines, so we need to consider the several international efforts at harmonization and regulation.

Most guidelines agree that the following steps are necessary to comply with the FDA regulations for drugs:

1. Identification of the analyte: qualitative analysis
2. Method development: quantitative analysis. Composed of an assay method for the major component and the determination of impurities and/or degrandants
3. Method validation
4. Method transfer (when necessary)
5. Stability testing

Steps 2 and 3 will be discussed after we take a look at the available guidelines.

Sources of Guidelines

This chapter has highlighted the guidelines and guidances from the U.S. FDA and its related agencies. However, other organizations have also been active in producing international guidelines. In July 1993, representatives of IUPAC and ISO met in Washington, D.C., for the purpose of developing common concepts and terminology.[42] The IUPAC recommendations resulting from that meeting were published in 1995 and have been reprinted in 1999[43] in an issue of *Analytical Chimica Acta* devoted completely to new recommendations related to validation.[44]

The ISO's publication on requirements for competence of testing and calibration was mentioned earlier,[24] but it contains only general guidelines much like the FDA's GMPs and the general ICH documents. For specific information one must consult ISO guides or other collaborative documents such as those discussed in the next section.

Of importance in international circles is the CITAC/Eurachem guide to quality in analytical chemistry.[45] This 57-page document can be downloaded from the CITAC web site and is an updated version of a joint effort between CITAC and Eurachem, newly revised in 2002 to incorporate ISO 17025. Another of their joint publications concerns analytical measurements and statistics.[46] It too is a valuable document (120 pages) and can be downloaded from the Internet. Eurachem's guide to method validation,[47] while intended for European analysts, is relevant for analysts in the United States, too. Another international document that resulted from a 1996 meeting co-sponsored by IUPAC, AOAC International, and ISO concerns recovery information.[48] One final source that must be included is the Organization for Economic Cooperation and Development (OECD) series on *Principles of Good Laboratory Practice and Compliance Monitoring*.[49] It too can be downloaded from the OECD web site.

Method Development, Validation, and Transfer

Many aspects of method validation have already been mentioned; it is the most important part of assuring that an analytical test method is suitable for its intended purpose. In this section we will look at the processes of method development and validation that proceed together as a new method is designed. Following that general discussion, some of the specifics regarding chromatographic method development and validation will be discussed.

Although there are now many publications on method validation, one that many find useful is Green's "A Practical Guide to Analytical Method Validation."[50] After a brief introduction, he proceeds through the recommended steps starting with "establish minimum criteria." Most of the subsequent steps describe the procedures for meeting the criteria established in the ICH document on validation, Q2A (see Appendix A). Some of them are discussed below.

Notice of the official FDA guidance document regarding method validation for new drug applications (NDAs) was first published in August 2000.[51] The highlights have been presented in a short study by FDA chemists.[52] Useful discussion of validation is contained in the series of articles entitled *Validation Viewpoint* by Krull and Swartz in *LC-GC*, and in their book on the topic.[53] For example, they discuss specificity in their June 2001 column[54] and validation of impurity methods in two later columns.[55, 56] Another recent work discusses validation following the ISO protocols and provides a good comparative discussion and useful ISO references.[57] Also, the USP General Chapter 1225 covers method validation in a way that generic drug manufacturers find useful. Originally published in the 1980s, the current version incorporates much material from the two ICH guidelines, but there are some differences.

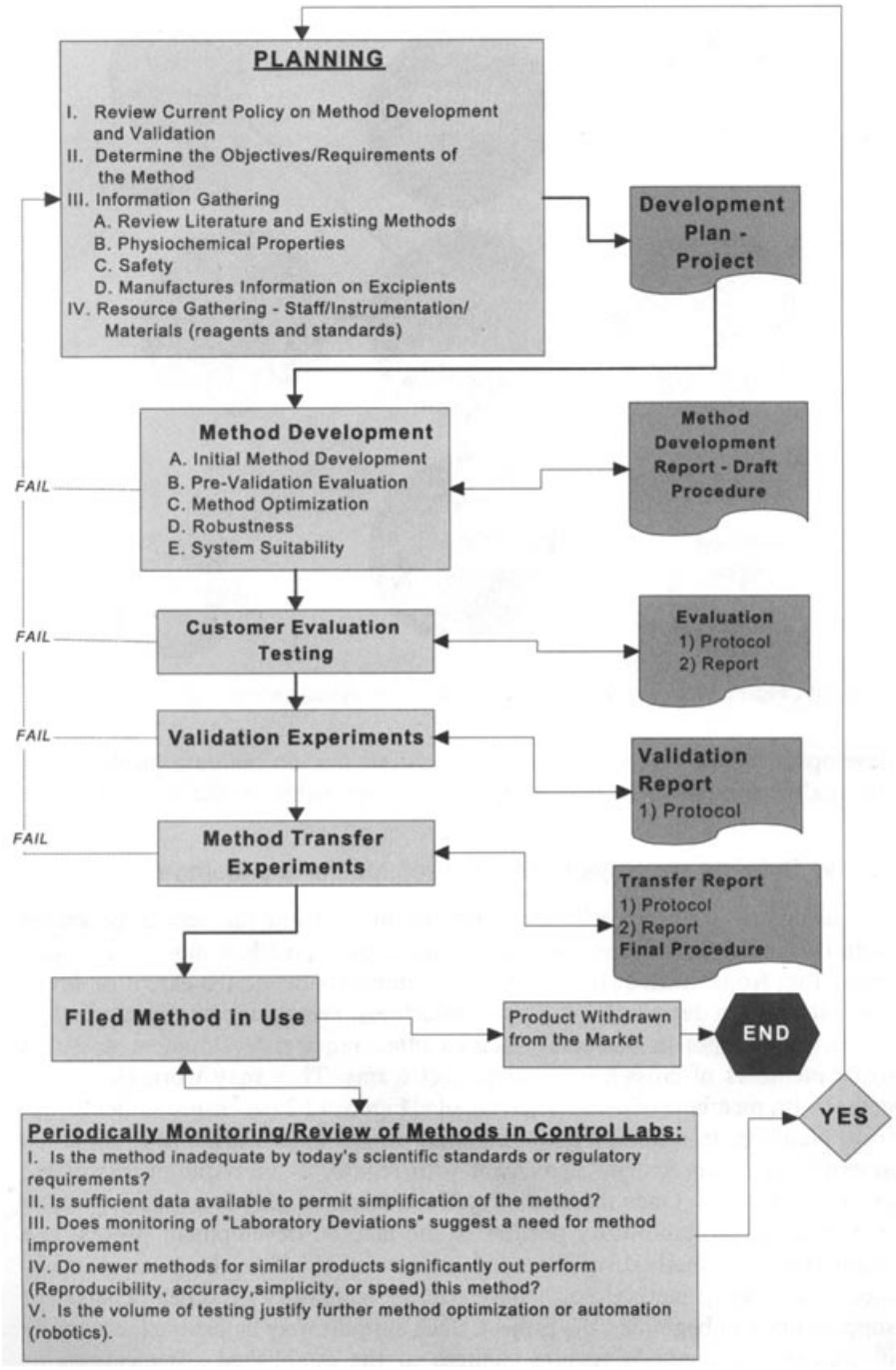

Figure 1.3. A pharmaceutical method development flowchart. Reprinted with permission from J. Miller and J. Crowther (eds), *Analytical Chemistry in a GMP Environment*, Copyright 2000; this material is used by permission of John Wiley & Sons, Inc.

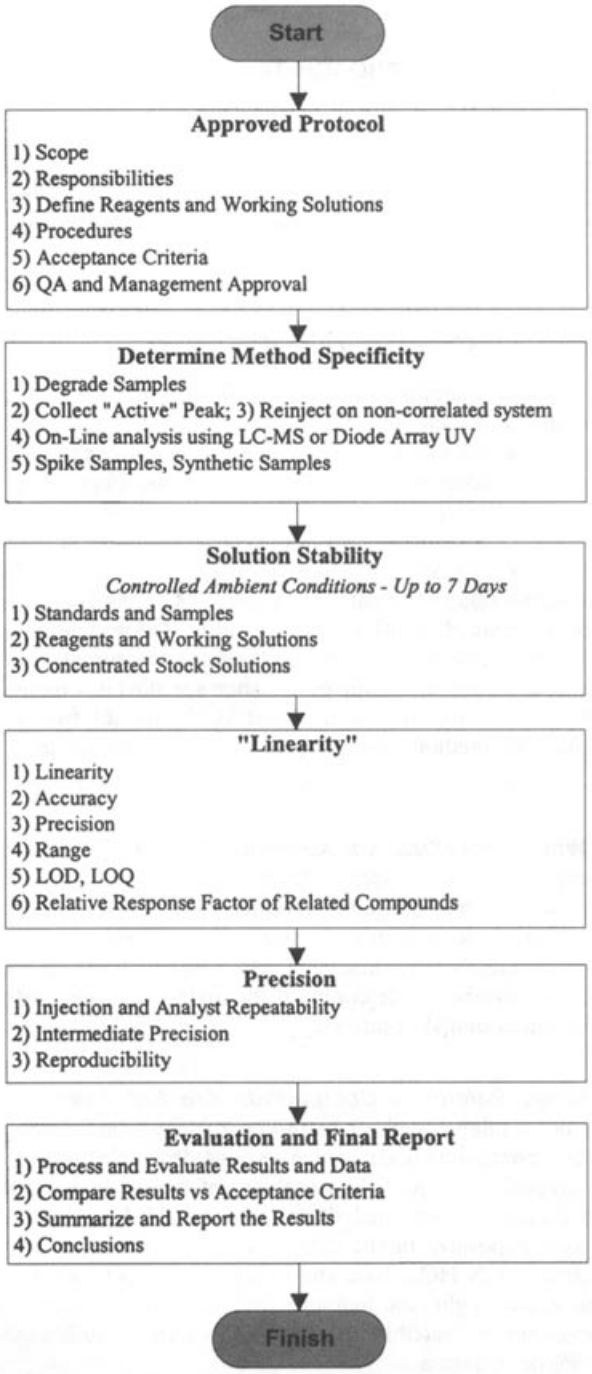

Figure 1.4. A validation process for an HPLC assay/purity method. Reprinted with permission from J. Miller and J. Crowther (eds), *Analytical Chemistry in a GMP Environment*, John Wiley & Sons. Copyright 2000; this material is used by permission of John Wiley & Sons, Inc.

An extensive diagram of the validation process is shown in Figure 1.3 (from reference 11), which has been nick-named V-TR^{2}AP, which stands for a developmental approval process that is intended to yield methods that are validatable, transferable, robust, reliable, accurate, and precise—another list of five criteria similar to the ICH list. Figure 1.4 is a schematic of a validation process specifically for an HPLC assay or purity method. Much more information can be found in the original reference.[11] For additional information on HPLC validation see references 56–58.

Some of the specific FDA guidelines taken from the ICH document on *Validation of Analytical Procedures*[61] are as follows:

- *Range* "If assay and purity are performed together as one test, and only a 100% standard is used, linearity should cover the range from the quantitation limit (QL) or from 50% of the specification of each impurity, whichever is greater, to 120% of the assay specification."
- *Accuracy* "Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each)."
- *Precision* "Repeatability should be assessed using: (a) a minimum of 9 determinations covering the specified range for the procedure (3 concentrations/3 replicates each) or (b) a minimum of 6 determinations at 100% of the test concentration."
- *Detection limit* (*DL*) "A signal-to-noise ratio (S/N) between 3 or 2:1 is generally acceptable. The DL may be expressed as

$$\mathrm{DL} = 3.3\frac{\sigma}{S} \tag{1.1}$$

  where σ = the standard deviation of the response and S = the slope of the calibration curve."
- *Quantitation limit* (*QL*) "A typical signal-to-noise ratio is 10:1. The QL may be expressed as

$$\mathrm{QL} = 10\frac{\sigma}{S} \tag{1.2}$$

  Further details are available in reference 59 and in several brief but informative articles."[62, 63]

Obviously, this list does not include all the definitions in the ICH document.[61] For example, there is no specific recommendation for robustness; rather, reference is made to ICH documents Q2A and Q2B. In such situations, it is up to the individual organization or company to write its own specification, usually in the form of a standard operating procedure, or SOP. Published reports can be consulted for information about the experiences of

other laboratories, such as these on robustness of HPLC methods.[64, 65] Other laboratory practices such as the frequency of calibration of laboratory balances should be specified in SOPs in accordance with the general principles of the GXPs. The FDA is willing to accept a reasonable specification or practice in cases where there are no specifics in its guidances. Written SOPs are required, and evidence must be available to show that they have been followed.

The ICH guidance document[61] also says that all chromatographic analytical procedures should include system suitability (SS) testing and criteria. This term is not in the ICH glossary in the appendix and deserves further discussion. As the name implies, SS is the process of demonstrating that a (chromatographic) system is functioning properly (is suitable) and is ready for use. The USP lists the SS criteria for GC and LC as: the precision (relative standard deviation, RSD) from five injections if the RSD is 2.0% or less, six injections if the RSD is greater than 2.0%; the resolution R_S; and the tailing factor, T. More extensive recommendations are given in the CDER *Guidance on Validation of Chromatographic Methods*,[15] which states that:

1. Capacity factor (retention factor) should be greater than 2.
2. RSD of $\leq 1\%$ for $n \geq 5$ is desirable.
3. $R_S \geq 2$.
4. $T \leq 2$.
5. Plate number ≥ 2000.

(These terms are defined in Chapter 2).

We have already noted many times that there is no universal set of guidelines and recommendations, and this is also true of those we have just presented. Those of the FDA and the ICH are representative and are probably the most often used and quoted in the United States. Two others that should be read are the ISO[28] and the CITAC/Eurachem guides.[45]

Two new alternative definitions of detection limit and quantitation limit that should be noted are currently under revision by the EPA.[66] They are slightly different from the ICH/FDA recommendations, and no mention of the latter is included in this new EPA proposal. To quote from the recent EPA document:[66]

> EPA focused its assessment on four sets of concepts that are widely referenced and generally reflect the diversity of concepts advanced to date. These include
>
> (1) The EPA MDL [minimum detection limit] and ML [minimum level of quantitation] used under the CWA [Clean Water Act] programs,
> (2) the Interlaboratory Detection Estimate (IDE) and Interlaboratory Quantitation Estimate (IQE) adopted by ASTM International,

(3) the Limit of Detection (LOD) and Limit of Quantitation (LOQ) adopted by the ACS, and (4) the Critical Value (CRV), Minimum Detectable Value (MDV) and Limit of Quantitation (LOQ) adopted by the IUPAC and the ISO.

Although the ACS, IUPAC, and ISO concepts are functionally similar to EPA's MDL and ML, these organizations have not developed detailed procedures for calculating detection and quantitation values. Only the EPA and ASTM concepts are supported by detailed procedures for calculating detection and quantitation values. Without such procedural details, the ACS, IUPAC, and ISO concepts are unlikely to be useful for establishing detection and quantitation limits in analytical methods for use in CWA programs. Therefore, the discussion below addresses the EPA and ASTM concepts only.

The proposed EPA definition of minimum detection limit (MDL) is

$$\text{MDL} = s \times t \tag{1.3}$$

where s is the standard deviation of the results and t is the Students t value from statistical tables for 99% confidence level and $(n-1)$ degrees of freedom. Similarly, the definition of minimum level of quantitation (ML) is

$$\text{ML} = 10s \tag{1.4}$$

but because the standard deviation, s, may not be readily available, the ML is often calculated from the MDL. Assuming a sample size of 7 (the minimum recommended by EPA), the MDL becomes

$$\text{MDL} = 3.143 \times s \tag{1.5}$$

and

$$\text{ML} = \frac{(10\ \text{MDL})}{3.143} = 3.18 \times \text{MDL} \tag{1.6}$$

For larger number of samples, the constant multiplied by the MDL will increase slightly; for example, for $n = 10$, the multiplier is 3.54.

Unfortunately, the symbols and the equations of the EPA and ICH are slightly different so there are no universal international standards for these two parameters, but they are close and one can hope for greater harmonization in the years to come.

Instrument Qualification

Instrument qualification is the process of making sure an instrument is performing properly. Usually it is accomplished in four stages called (by GAMP 4[67]): design qualification (DQ), installation qualification (IQ), which

may be performed by the instrument manufacturer, operational qualification (OQ), and performance qualification (PQ). A certified standard, such as an SRM from NIST, should be used where applicable. Details are on the International Society for Pharmaceutical Engineering (ISPE) web site.[68] Quite a few brief articles have been written on instrument qualification,[69, 70] including one specifically on performance qualification of LC systems.[71]

Once an instrument passes these tests, it is ready for use. In addition, during its use, the instrument should be properly maintained, and at some later time be recalibrated. An SOP should be written to designate the time intervals and the procedures for accomplishing calibration as well as any preventative maintenance that may be required. In some cases, the PQ can serve as the basis for the recalibration procedure.

21 CFR Part 11: Electronic Records and Electronic Signatures

Previously, chromatographic raw data could be easily defined as a piece of chart paper containing a particular chromatogram. The definition is much more complex when the data are digitally recorded in a computer data file. The regulation that is concerned with these issues is referred to as 21 CFR Part 11. That name specifies that the regulation can be found in Part 11 of Section 21 of the CFR. It deals with the tracking of computerized data, including keeping records of those with access to it and tracking the changes they have made in processing the data. This topic is receiving considerable attention at present in an attempt to arrive at final FDA regulations. Further information about this subject has been published in many journals (see, e.g., references 72 and 73) and the latest FDA (draft) guidance document was published in February 2003.[74] It contains the references to the five previous guidance documents on this subject and withdraws the previous guidance and the Compliance Policy Guide 7153.17. A new guidance was issued on September 4, 2003, announcing that the FDA intends to exercise discretion in enforcing some requirements of Part 11 while it reexamines the regulation.[75]

1.7 FINAL COMMENTS

Although this discussion has focused on the FDA regulations, it should serve to introduce chromatographers to the complexities of the current industrial practices and recommendations. More discussion is included in Chapter 2 (definitions and symbols) and Chapter 9 (quantitative analysis). Additional information about practices in the pharmaceutical industry can be found in references 11 and 12.

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