

Standard Practice for Use of Reversed-Phase High Performance Liquid Chromatographic Systems¹

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1. Scope

1.1 This practice covers requirements for defining, testing, and verifying the performance of high performance liquid chromatographic (HPLC) systems when used for trace analysis of pesticides and toxic substances in carrying out pollution control programs and in assessing the quality of food products as mandated by national laws and regulations. As a practical matter, this practice is intended to cover requirements of reversed-phase (adsorption) HPLC systems. Microbore column and ion-exchange column HPLC systems are not covered here. It is not intended to exclude any other equivalent means of analysis. An HPLC system can successfully be applied in the analysis of a variety of sample types including ground and surface water, municipal and industrial effluents, workplace air, soils and sediments, plant and animal tissue, and food products **(1, 2, 3)**.² Collection and extraction techniques, appropriate to the sample type, are required prior to analysis. Sampling techniques and measurement methods are not covered in this recommendation; however, some relevant measurement methods may be found in references listed in Appendix X1.

NOTE 1—High performance liquid chromatography is synonymous with high pressure liquid chromatography.

1.2 Metrological and technical requirements are provided for the major components of an HPLC system including the pump(s), injector(s), column(s), detector(s), and temperature control and data handling systems. The conditions of operation of a single integrated instrument, or one combined from separate components, are intended to cover the application for trace analysis.

1.3 Basically four types of packed columns for liquid chromatography exist: partition, adsorption, ion exchange, and gel permeation. Other terms are used to refer to each type. For many separations, however, the actual separation may not be clearly defined and may involve a combination of retention mechanisms. Furthermore, the polarity of the stationary phase

can be greater or less than the mobile phase. The separation method is called normal-phase HPLC when the stationary phase is more polar than the mobile phase, and the separation method is called reversed-phase HPLC when the reverse condition exists. The reversed-phase HPLC system, using an adsorption column, has become the more frequently used technique for separation and analysis of organic compounds. It can separate a broad spectrum of nonionic, ionizable, and ionic compounds, and its columns are usually stable and separations may be performed with good repeatability since the stationary phases are chemically bonded.

1.4 The detector type selected for use with an HPLC system depends generally on the concentration as well as the chemical and physical properties of the sample matrix and the analyte to be measured. The following detectors are covered in this practice. UV/Visible spectrophotometric, fluorescence, electrochemical, and refractive index.

NOTE 2—The mass spectrometer is a highly specific and sensitive detector appropriate for most applications. It is normally coupled to the HPLC system through an appropriate interface. The use of a mass spectrometer as a detector is not covered in this practice because of its specialized nature.

1.5 The following are examples of classes of analytes that may be measured by an HPLC system: carbamates, pyrethroids, organophosphates, polycyclic aromatic hydrocarbons, phenolics, isocyanates, aflatoxins, chlorophenoxy-acid herbicides, triazine herbicides, and amines. An advantage of HPLC over gas chromatography is that it may be used for the direct measurement of thermally labile compounds, compounds of low volatility, and strongly polar compounds without conversion to derivatives.

1.6 Optimizing the performance of each major component of the measuring system may achieve performance better than the criteria prescribed for these applications. Success in this respect depends on the knowledge, skill, and experience of the analyst.

1.7 The values stated in SI units are to be regarded as the standard.

¹ This practice is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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² The boldface numbers given in parentheses refer to a list of references at the end of the text.

