ORGANISATION INTERNATIONALE

DE MÉTROLOGIE LÉGALE

OIML INTERNATIONAL RECOMMENDATION

High performance liquid chromatographs
for measurement of pesticides and other toxic substances

Chromatographes en phase liquide de haute performance pour la mesure des pesticides
et autres substances toxiques

OIML R 112

Edition 1994 (E)

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FOREWORD

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This publication - reference OIML R 112, edition 1994 (E) - was developed by the OIML subcommittee TC 16/SC 3 «Instruments for measuring pollutants: pesticides and other toxic substances». It was approved for final publication by the International Committee of Legal Metrology in 1993 and will be submitted to the International Conference of Legal Metrology in 1996 for formal sanction.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHS
for MEASUREMENT of PESTICIDES
and OTHER TOXIC SUBSTANCES

1 Scope

1.1 This Recommendation provides requirements for defining, testing, and verifying the performance of high performance liquid chromatographic (HPLC) systems when used for measurement 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. It does not intend to exclude any other equivalent means of measurement and analysis. An HPLC system can be applied successfully 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, and 3]. Sample collection and extraction techniques, which depend on the sample type, are required prior to analysis. Sampling techniques and measurement methods are beyond the scope of this Recommendation; however, some relevant measurement methods may be found in references listed in Annex A.

Note: 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 instrument, or one combined from separate components, are intended to cover the application for trace analysis.

1.3 Four principles of separation in 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 mechanism may not clearly be defined and may involve adsorption, partition, or a combination of both. 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 has become the more frequently used technique for separation and analysis of organic compounds. It can separate a broad spectrum of non-ionic, ionizable, and ionic compounds. Reverse phase columns are usually stable and separations may be performed with good repeatability since the stationary phases are chemically bonded. Therefore, this Recommendation is intended to cover requirements of reversed-phase HPLC systems. Microbore-column, ion-exchange-column, and gel-permeation-column HPLC systems are not covered in this Recommendation.

1.4 The detector type selected for use with an HPLC system depends generally on the concentration and chemical and physical properties of the sample component to be measured and its matrix. The following detectors are covered in this Recommendation: UV/visible spectrophotometric, fluorescence, and electrochemical.

Note: The mass spectrometer is a highly specific and sensitive detector appropriate for application. It is normally coupled to the system through an appropriate interface and is to be covered by a separate Recommendation because of its specialized nature.

1.5 The following are examples of compound classes that may be measured by an HPLC system: carbamates, pyrethroids, organophosphates, polynuclear aromatics, phenolics, isocyanates, aflatoxins, chlorophenoxy-acid herbicides, triazine herbicides, and amines. Some sample components may have to be converted to derivatives before measurement; however, an advantage of an HPLC system is that it may be used for the direct measurement of thermolabile compounds, compounds of low volatility, and strongly polar compounds without conversion to derivatives.

1.6 Instrument performance better than the criteria prescribed for these applications may be achieved by optimizing the performance of each major component of the measuring system. In such cases, success may depend on the knowledge, skill, and experience of the analyst.

2 Terminology

Note: References [2], [4], and [5] provide definitions of terms relevant to this Recommendation. The definition of some terms presented here, however, have been modified for better interpretation of this application.

2.1 Injection device for the instrument

The means of introducing a sample into the column.

2.2 Column

A tube that contains the stationary phase through which the mobile phase flows.

2.3 Mobile phase

The liquid (solvent) used to elute the sample components through and from the column; it may consist of a single component or a mixture of components.

2.4 Stationary phase

The active immobile material within the column attached to the solid support or the solid support itself that delays the passage of sample components by one of several possible processes or by a combination of such processes.

2.5 Solid support

The material within the column to which the stationary phase is bonded (together comprising the packing material) and through which the mobile phase flows. Ideally this material is inert. It may be characterized by the particle diameter d_p measured in µm.

2.6 Elution

The removal of a sample component from the stationary phase by the mobile phase.

2.7 Detector

The device that responds to the presence of sample components eluting from the column.

2.8 Noise

A measure of variation in the detector signal; it can be divided into three components:

Short-term noise includes all observable random variations of the detector signal having a frequency of the order of 10^{-2} Hz to 10^{-1} Hz (one or more cycles per minute) and should be measured peak to peak.

Long-term noise includes all observable random variations of the detector signal with frequencies between 0.1 Hz and 1.0 Hz.

Note: Althoug long-term noise can occur at any time during an analysis, it can be mistaken for a late-eluting peak. Earlier eluting peaks are usually sharper and less easily confused with long-term noise.

Drift is the average slope of the baseline signal measured over a minimum period of one-half hour.

2.9 Detection limit for the instrument

The concentration of the sample component of interest that gives a detector output signal equal to three times the short-term noise.

Note: This term is also referred to as «minimum detectability» in some references and manufacturer's literature. It is sometimes defined as an output signal equal to some other multiple (for example, two or ten) of the noise.

2.10 Linear range of a detector

The range of concentrations or mass flow rates of a sample component of interest over which the sensitivity of the detector is constant to within 5 %. Its value is the ratio of the upper limit of linearity to the detection limit.

2.11 Dynamic range of a detector

The range of concentrations or mass flow rates of a sample component over which a change in sample amount produces a measurable change in the detector signal output. Its value is the ratio of the upper limit of detection to the detection limit.

2.12 Sensitivity of a detector

The output signal per unit concentration of the sample component in the mobile phase; it may be expressed as:

$$S = \frac{A \times F}{M}$$

where

S = sensitivity
A = the integrated area of the sample component
F = the flow rate
M = the mass of the sample component injected

Note: An electrochemical detector of the coulombic type does not follow this equation.

2.13 Chromatogram

A record of the detector output signal versus time that has specific peaks associated with measured components of a sample

2.14 Retention time (t_r) for a measurement

The elapsed time between injection of a sample and the appearance of the maximum output peak of the component of interest.

Note: A related term is capacity factor k', which is defined as follows:

$$k' = \frac{t_r - t_m}{t_m}$$

where

t_m = the time for the mobile phase to proceed from the point of injection to the point of detection.

2.15 Repeatability

The closeness of agreement between results of successive measurements of the same measurand carried out under the same conditions and within a relatively short period of time.

Note: The same conditions include the following: the method of measurement, the operator, the measuring instrument, the location, and the environmental conditions.

3 Description of the instrument

3.1 General

3.1.1 A diagram of an HPLC system is shown in Figure 1. The mobile phase from a solvent reservoir is filtered and then pumped through the injector, column, and detector, any one or all of which may be enclosed in a thermostatically controlled oven. A

sample is introduced through an injection device to the column and is then separated into its components while passing through the column. A guard column may be required to retain materials that could degrade the column performance. Similarly, a post-column reactor may be necessary for derivatization of sample components. The eluate is monitored by a detector that responds to sample components. The detector output signal is displayed instantaneously and/or stored in a data handling system. The eluate is finally collected in a waste container for proper disposal.

3.1.2 Separation of sample components by a column depends on the interaction of the column packing (stationary phase) with the mobile phase. Isocratic elution means that the composition of the mobile phase is kept constant, and gradient elution means that the composition of the mobile phase is deliberately changed during the chromatographic procedure. Gradient elution is the same, therefore, as mobile phase or solvent programming.

3.1.3 The individual sample components eluting from the column are monitored by a detector. The output signal versus time of the detector provides peak areas or peak heights that may be related to the concentration of sample components.

3.1.4 The overall performance of an HPLC system may be characterized by the repeatability of measurements of the retention time and peak height (or area) for specific sample components under controlled measurement conditions.

3.2 Major components

3.2.1 Mobile phase

The mobile phase has a composition and purity which depend on the method of analysis, detector used, and whether isocratic or gradient elution is employed. The mobile phases most commonly used in reversed-phase HPLC are mixtures of either methanol or acetonitrile with water.

3.2.2 Pumps

3.2.2.1 A syringe pump is used to move the mobile phase with a mechanically controlled piston advancing at a constant rate in a fixed volume chamber.

3.2.2.2 A reciprocating pump is used to move the mobile phase with one or more heads having small volume chambers with reciprocating pistons or diaphragms. Check valves are synchronized with the piston (or diaphragm) drive to alternate the filling and emptying of the mobile phase from each chamber.

Note: Mechanical pulse dampers and/or electronic or pneumatic transducers are often incorporated to ensure that the flow rate is constant.

3.2.3 Injection device

The injection device most commonly used is a valve and loop in which the sample contained in a syringe is introduced into a chamber, or loop, at ambient pressure and is subsequently displaced into the pressurized flowing mobile phase by means of a rotary or sliding motion.

Note: Septum and septumless injection devices may be used. The process of injection is often automated.

Injector

Guard
column

Oven

Column

Mobile phase
solvents

A   B

Pump

Data handling
device

Detector

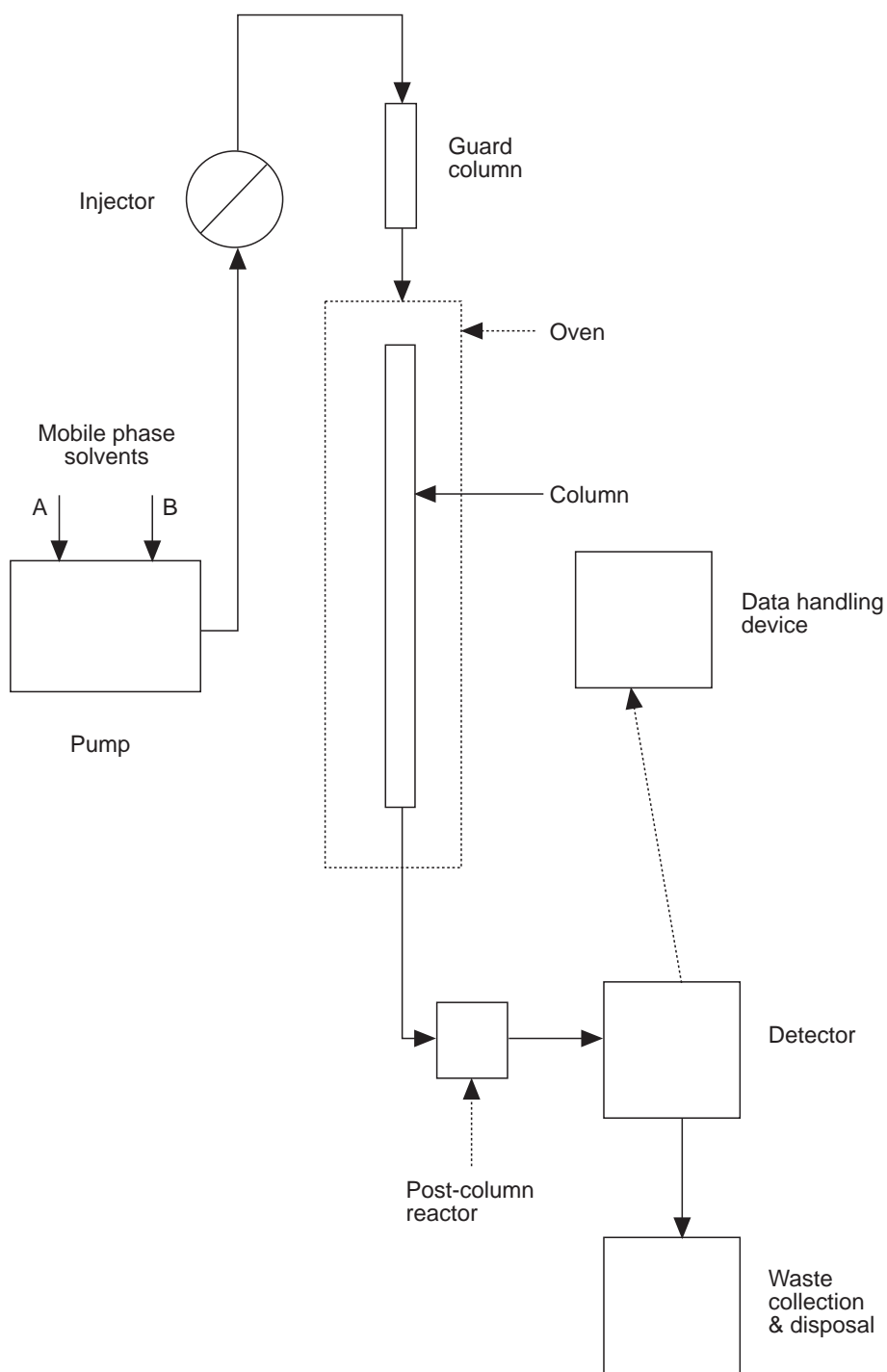Post-column
reactor

Waste
collection
& disposal

Figure 1

Schematic diagram of an HPLC system. Note that the guard column, column oven, and/
or post column reactor may be included, or excluded as required by the analytical method. The column
reactor may be located just ahead of the column and is then referred to as a «pre-column» reactor.

3.2.4 Guard column

A guard column may be placed between the injection device and column to protect the latter from loss of efficiency which may be caused by the presence of particulate matter or strongly absorbing material from the sample.

Note: A filter should be added if a guard column is not present.

3.2.5 Column

The column used depends on the analytical method. It should be selected for optimum separation of the sample components of interest.

Note: Bonded-phase silicas are widely used as packing (stationary phase and solid support). For reversed-phase HPLC, octadecylsilyl is the most commonly used stationary phase although alkyl, phenyl, and nitrile functional groups may also be used. Two types of packing are generally used: porous packing in which the stationary phase is found throughout each porous particle and pellicular packing in which the stationary phase is found only on the outer layer of an otherwise impermeable particle.

3.2.6 Reactor

A pre- or post-column reactor may be used to prepare derivatives of sample components of interest prior to detection.

Note: It is important to prevent backflushing of reagents either by using appropriate flowrates or pressures or by the use of check valves.

3.2.7 Detectors

3.2.7.1 UV/visible spectrophotometric detectors may be used for measuring the absorbance of light by the sample as it elutes from the column when the absorbance measured can be related to the concentration of the sample component of interest. Single (fixed) wavelength detectors are used most often with a low-pressure mercury light source and at wavelengths determined by the mercury emission lines and an optical filter. Appropriate variable wavelength detectors include a grating monochromator to select one or more wavelengths and a photodiode array with optics to select several wavelengths simultaneously. Deuterium, tungsten, or pulsed-xenon lamps are usually used as the light sources to cover the wavelength range for the variable-wavelength detectors.

3.2.7.2 Fluorescence detectors may be used to relate the fluorescence emission to the concentration of sample components of interest that have been excited by absorption of light. Fluorescence transitions result in band spectra since sample components are usually excited by a spectrum of wavelengths from a light source; however, the detector is often operated at a wavelength corresponding to the intensity maximum in the excitation and emission spectra for the sample component of interest in order to obtain maximum sensitivity and selectivity.

3.2.7.3 Electrochemical detectors may be used for measuring the concentration of sample components that can be oxidized or reduced electrochemically on electrodes. A cell current within the detector is produced by the eluate from the column. It is measured at a fixed potential and is directly proportional to the concentration of the affected sample component.

3.2.8 Data handling system

The data handling system provides a means for recording and displaying the output signal of an HPLC system detector as a function of time. A potentiometric strip chart recorder, computing integrator, and/or a computer system may be used for this purpose.

4 Metrological requirements

4.1 Pumps

4.1.1 The pump shall be capable of performing over a pressure range from 0 MPa to 40 MPa.

4.1.2 The pump shall be capable of delivering flow rates from 0.1 mL/min to 5 mL/min. When tested with water, its repeatability of flow rate shall be within ± 3 % as determined for a flow rate of 1 mL/min and collected over a ten-minute interval at an overall system pressure of at least 14 MPa.

Note: Pumps may be able to deliver flow rates greater than this range and still meet the requirement.

4.1.3 The pump's pulsation shall be less than ± 2 % peak to peak in pressure when monitored using water at a back pressure of 14 MPa and at a flow rate of 1 mL/min over a ten-minute interval. This measurement shall be carried out at the pump just before the injector and at the operating pressure.

Note: The pressure of the pulsations at the detector will be much less than that measured, since the detector is at the lowest pressure site of the system. Pump performance may affect the detection limit of a detector for some sample components; therefore, to optimize system performance, the detector noise generated by the pump should be minimized.

4.2 Injectors

Injectors shall be capable of delivering a specific sample volume with a repeatability of ± 1 % or ± 0.05 µL, whichever is greater, for a volume within the range from 1.0 µL to 1 000 µL. Injectors shall meet these requirements for pressures up to 34 MPa.

4.3 Column temperature

For a column with a means for temperature control, the column temperature shall be adjustable in 1 °C increments and regulated to within ± 0.1 °C.

Note: When an HPLC system column is operated at temperatures of 10 °C or more above ambient, preheating of the mobile phase may be required to avoid bubble formation.

4.4 Detectors

4.4.1 The detectors shall meet the minimum performance requirements for detection limit and linear range when tested according to the prescribed conditions of this subclause and those of Annexes B and C. The detection limit specified is considered to be the maximum permissible value for this quantity with maximum permissible errors of

± 10 %, and the linear range is considered to be the working range for quantification for the instrument although the manufacturer may specify a dynamic range within specified limits (see Annex C). The working ranges for temperature and humidity for the instrument are from 10 °C to 35 °C and 60 % ± 20 %, respectively.

4.4.2 The following general test conditions apply:

- Select a column with a packing according to the manufacturer's recommendation.

- Select the mobile phase and column temperature recommended by the manufacturer using isocratic elution.

- Select an injector of an appropriate size and type for the amount of test compound to be injected.

- Control the temperature of the detector to within ± 1 °C at a specific temperature in the working temperature range of the instrument or usually between 10 °C and 23 °C.

4.4.3 UV/visible spectrophotometric detector

- Type: concentration and cell length dependent, selective and widely applicable

- Test compound: anthracene

- Wavelength: 254 nm ± 5 nm fixed nominal range

  200 nm ± 10 nm to 600 nm ± 10 nm variable nominal range tuned at 252 nm ± 2 nm

- Test conditions: the conditions shall be compatible to those in Annex B

- Output peak width at half height $\leq 2 \times \left[5 \times \dfrac{t_\mathrm{r}^{\,2}}{L} \times d_\mathrm{p} \right]^{1/2} \times 0.01$ min

  for a signal to noise ratio greater than 100
  where
  t_r = retention time
  d_p = particle diameter in µm of the packing of the column and
  L = length of the column in cm

Note: Values for particular column specifications are provided in B.3.6.

- Detection limit: 3 ng anthracene injected (± 2 %)

- Linear range: 10^4

Note: Additional details on these tests are given in Annex C and reference [6].

4.4.4 Fluorescence detector

- Type: concentration dependent, selective and compound specific cell volume dependent

- Test compound: anthracene

- Wavelength: 250 nm ± 5 nm excitation maximum

  400 nm ± 5 nm emission maximum

- Test conditions: the conditions shall be compatible to those in Annex B

- Output peak width at half-height: the requirements shall be the same as those specified in 4.4.3
- Detection limit: 1 ng anthracene injected (± 10 %)
- Linear range: 10^3

Note: Additional details on these tests are given in Annex C.

4.4.5 Electrochemical detector

- Type: concentration dependent, selective

- Test compound: hydroquinone (oxidation)
                       quinone (reduction)

- Test conditions: the conditions shall be compatible to those in Annex B

- Output peak width at half-height $\leq 4 \times \left[5 \times \dfrac{t_r^2}{L} \times d_p \right]^{1/2} \times 0.01$ min

  for a signal to noise ratio greater than 100

- Detection limit: 50 pg hydroquinone injected (± 10 %)

  Note: The sensitivity of the detector depends on the flow rate in the detector cell (see 2.12 and Annex C)

- Linear range: 10^3 to 10^4

5 Technical requirements

5.1 The mobile phase should contain reagent (HPLC) grade chemicals which may require further purification for some measurements. The water used shall be of high purity especially with respect to organics. Before use, the mobile phase shall be filtered using the filter porosity and media recommended by the instrument manufacturer. It shall also be degassed when necessary to prevent bubble formation in the HPLC system.

5.2 A pressure monitor and a pressure limit switch shall be provided in the system between the pump and the column.

5.3 The pump shall be designed to deliver a single mobile phase at a constant rate through the column for isocratic elution. For gradient elution, solvent gradients may be generated either by a controlled mixing of the mobile phase components on the inlet side of a single pumping system or by combining the outputs of two or more pumping systems prior to the injector.

Note: In some gradient elution applications it may be necessary to premix solvents to prevent bubble formation and excess cooling when further controlled mixing occurs during the cycle.

5.4 Additional filters of from 2 µm to 10 µm may be used before the pump. A guard column or a filter should be used after the pump and the injection device in order to remove particulate matter.

5.5 The columns shall be tubular and packed; shall be constructed of metal, glass, or plastic; and shall be capable of withstanding the pressure generated by the pump.

Note: The column connections should be designed for easy assembly and disassembly.

5.6 All plumbing connections of the HPLC system shall be arranged so as to minimize the pre- and post-column volume («dead volume») of the system.

Note: The connections should be selected for compatibility in sealing against high-pressure fluids and for their resistance to dissolution or corrosion by the applicable mobile phase and any reagents to be used.

5.7 A means shall be provided for temperature control of the detectors if necessary to meet the requirements of 4.4.2.

Note: A metal block for a heat sink or a resistance type heater are examples of devices used for temperature control.

5.8 The data handling system shall have a means of accurately recording and displaying the output signal of an HPLC system detector as a function of time.

5.9 Markings shall be attached conspicuously to all major components of the HPLC system as follows:

• name of manufacturer

• instrument serial and model number and date of manufacture

• voltage, frequency, and current requirements

Note: Labels or declarations concerning personnel safety and radio frequency interference emission should be provided according to national regulations.

6 Practical instructions

6.1 HPLC systems use high voltage and flammable and potentially toxic liquids under high pressure during normal operation. Warning labels shall be conspicuously placed on the instrument to alert the user to these and other potential hazards. Instrument installation and operation, particularly with respect to ventilation and a means for disposal of sample and solvent wastes, shall be consistent with national hazardous waste disposal and safety regulations.

6.2 Manufacturers of HPLC systems or their components shall supply a manual that describes the installation, operation, and routine maintenance of the systems or components (see also reference [7]). Service and analytical methods manuals may be available upon request.

6.3 Before installation, all laboratory environmental factors shall be considered. Manufacturers shall provide potential users operating specifications for the HPLC system that include the power consumption, the upper and lower rated voltage and frequency, and the range of ambient temperature and humidity.

7 Metrological controls

7.1 Pattern Approval

7.1.1 Pattern approval testing shall provide means for attesting the conformity of a sample, or samples, of an instrument's pattern, or type, to the requirements of this Recommendation.

7.1.2 The manufacturer shall provide the responsible national body with the required number of instruments and an operating manual. In addition, manufacturer's test data, calibrations, and other relevant information which contribute to determining whether an instrument's pattern meets the requirements of this Recommendation may be provided.

7.1.3 The operating manual of the instrument shall be reviewed for completeness and clarity of instructions.

7.1.4 The responsible national body shall visually inspect the instrument in conjunction with the review of its operating manual to determine that the requirements are met for the following:
- pressure monitor and limit switch (5.2)
- pump and plumbing system (5.3 and 5.6)
- temperature control of detectors (5.7)
- data handling system (5.8)
- markings (5.9)

7.1.5 The responsible national body shall carry out tests, or may accept manufacturer's test data, that confirm the conformity of the following to the applicable performance requirements:
- pump (4.1.2 and 4.1.3)
- injection device (4.2)
- temperature control (4.3)
- detection limit (4.4 and Annex B)
- linear range and sensitivity of the detector (Annexes B and C)

7.1.6 The report on instrument tests carried out during pattern evaluation should contain at least the information according to the format specified in Annex D. A specific form may be developed according to national preference. The manufacturer shall be provided with specific comments about any test failures.

7.2 Initial and subsequent verification

7.2.1 Traditional legal initial and subsequent verification may not be practical for these instruments. However, the responsible national body should consider adopting the control procedures specified in 7.3 as a means of assuring the continued metrological integrity of an HPLC system.

7.2.2 Such control procedures may be established for specific analytical methods and may include a means for assessing laboratories using HPLC systems. Appropriate assessment procedures may include the following: accreditation of the user laboratory, self certification by the user laboratory, and proficiency testing through intercomparisons of measurements among user laboratories.

7.3 User tests and records

7.3.1 An initial test of an HPLC system shall be performed according to the manufacturer's instructions. The results of this test shall be within the specifications provided by the manufacturer.

7.3.2 Routine tests shall be carried out to determine and maintain the optimum operating conditions of the instrument for a particular analytical method. Reference materials should be used daily along with quality control charts to verify the working range of the instrument over an extended period of time. A routine procedure for checking the operational readiness of an HPLC system is given in Annex E.

Note: Appropriate reference materials may be available as indicated in references [8], [9], and [10].

7.3.3 An overall test of the entire HPLC system should be carried out frequently (for example, once per work period) using reference standards that are appropriate for the analytical method for the class of sample compounds. This test should specifically characterize the instrument's detection limit, sensitivity, and operating range. A performance test for an entire HPLC system is given in Annex C.

7.3.4 Published procedures by the responsible national body should specify performance tests, calibration procedures, and routine tests for HPLC systems appropriate for specific pollutants. The time interval between tests should be specified as appropriate.

7.3.5 Records shall be maintained containing the following written information in chronological order for each HPLC system:
- results of initial and overall performance test
- results of routine tests that document the retention time, peak area or height, and peak width
- identification of the reference sample for each analysis performed, of the mobile phase(s), injector(s), column(s) (including particle diameter), elution procedure, detector(s), and data handling system(s)
- description of malfunctions and corrective action taken
- extent of maintenance and/or repair

REFERENCES

[1] Poole, Colin F. and Schuette, Sheila A., *Contemporary Practice of Chromatography,* Elsevier, New York (1984).

[2] Standing Committee of Analysts, Department of the Environment, U.K., *High Performance Liquid Chromatography, Ion Chromatography, Thin Layer and Column Chromatography of Water Samples,* Her Majesty's Stationery Office (1983).

[3] Lawrence, James F., Editor, *Liquid Chromatography in Environmental Analysis,* Humana Press, Clifton, New Jersey (1984).

[4] Joint Working Group of BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, and OIML, *International Vocabulary of Basic and General Terms in Metrology,* ISO (1993).

[5] ASTM E682-79, Practice for *Liquid Chromatography Terms and Relationships,* Annual Book of Standards, Vol. 14.01, ASTM, Philadelphia (1985).

[6] ASTM E685-79, Practice for *Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography,* Annual Book of Standards, Vol. 14.01, ASTM, Philadelphia (1989). ISBN 0-8031-1367-6

[7] Runser, D.J., *Maintaining and Troubleshooting HPLC Systems,* John Wiley and Sons, New York (1981).

[8] International Organization for Standardization, *ISO Directory of Certified Reference Materials,* (Current Directory) Geneva, Switzerland.

[9] United States Environmental Protection Agency, Quality Assurance Division, Environmental Monitoring Systems Laboratory, Office of Research and Development, *Analytical Reference Standards Supplementary Data:* Pesticides and Industrial Chemicals, Repository, EPA-600/4-84-082, (Current Catalog) Cincinnati, OH 45268, USA.

[10] United States National Institute of Standards and Technology, NIST Standard Reference Materials Catalog, *NBS Special Publication 260,* (Current Catalog), Gaithersburg, MD 20899, USA.

[11] Karger, B.L., Snyder, L.R., and Horvath, C., *An Introduction to Separation Science,* pp. 135-138, Wiley Interscience, John Wiley and sons, Inc., New York (1973). ISBN 0-471-45860-0.

ANNEX A

REFERENCES TO MEASUREMENT METHODS

A.1 U.S. Food and Drug Administration (FDA), *Pesticide Analytical Manual.*

A.2 U.S. Environmental Protection Agency, *Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act: Final Rule and Interim Final Rule and Proposed Rule,* 40 CFR Part 136, U.S. Federal Register Notice, Friday, October 26, 1984.

A.3 Association of Official Analytical Chemists, *Official Methods of Analysis of the Association of Official Analytical Chemists,* Methods Manual, 14 Edition (1984), Arlington, VA 22209, USA.

A.4 United Kingdom Health and Safety Executive (HSE), *Methods for Determination of Hazardous Substances,* St. Hugh's House, Stanley Precinct, Bootle L20 3QZ, England.

A.5 APHA-AWWA-WPFC, American Public Health Association, *Standard Methods for the Examination of Water and Wastewater,* 16th Edition, (Revised every three years).

A.6 ASTM, Annual Book of Standards, *Index,* Vol. 00.01, (Current Edition).

A.7 International Organization for Standardization (ISO), *Catalogue,* (Current Edition).

ANNEX B

TEST CONDITIONS FOR HPLC DETECTORS
(Mandatory)

B.1 This Annex provides detailed tests for the detectors covered in 4.4.

B.2 UV/visible spectrophotometric detector and fluorescence detector

B.2.1 The nominal flow rate of the mobile phase for a selected internal diameter (I.D.)
of a column should be as follows:

I.D. (mm)	Flow rate (mL/min)
4.6	1.0
3.9	0.7
2.0	0.2

Note: For other I.D.s, use a flow rate = $(I.D.)^2/21.2$ mL/min.

B.2.2 The retention time for the test compound for a selected length of column should
be as follows:

Column length (cm)	Retention time range (min)
30	10.6 - 14.0
25	8.8 - 11.7
20	7.0 - 9.3
15	5.3 - 7.0
10	3.5 - 4.7
7.5	2.7 - 3.5
6	2.1 - 2.8

Note: The retention time reflects conditions that would give a capacity factor value of
between 2 and 3 (see 2.14). The holding time, t_m, is approximately 0.7 times the
volume of the column (i.e., length times π times the radius squared). This re-
flects common practice when measuring these parameters.

B.3 Derivation of the equation for output peak width.

B.3.1 The detector performance with regard to output peak width depends mainly on
the following variables: retention time (t_r), column length (L), and column packing-
particle diameter (d_p). In addition, the flow rate has an effect that has been taken into
account by specifying the flow rate for the nominal internal diameter of the column to
be selected (see B.2.1).

B.3.2 Consider the following basic equations:

$$N = \left[\frac{t_r}{\sigma_t}\right]^2 \; ; H = \frac{L}{N} \; ; h = \frac{H}{d_p}$$

where
N and H = measures of the efficiency of the column in separating components of a
sample (that is, the narrowness of peaks).
h = the reduced plate height
σ_t = the standard deviation of the peak

B.3.3 Using the equations of B.3.2, the following equation is derived for the standard deviation of the peak:

$$\sigma_t = \left[h \times d_p \times \frac{t_r^2}{L} \right]^{1/2}$$

B.3.4 Assuming that the peak width at half height can be approximated by multiplying 2, then the standard deviation becomes the following:

$$\text{Peak width at half height} \simeq 2 \left[h \times d_p \times \frac{t_r^2}{L} \right]^{1/2}$$

B.3.5 Commercial columns should have a reduced plate height (h) equal to 5 or smaller; that is, the closer h is to unity then the better is the performance of the column. The equation of B.3.4, therefore, reduces to the following:

$$\text{Peak width at half height} \simeq 2 \left[5 \times d_p \times \frac{t_r^2}{L} \right]^{1/2} \times 0.01 \text{ min}$$

where L is expressed in cm and d_p in µm.

Note: For more details concerning this derivation, see reference [11].

B.3.6 Using the expression in B.3.5, the average of at least three repeated measurements of maximum output peak width at half height for the test compound for various packing particle diameters and various lengths of a column should be the following:

column length (cm)	t_r (min)	3 µm (min)	5 µm (min)	10 µm (min)
30	12.3	0.17	0.22	0.32
25	10.2	0.16	0.20	0.29
20	8.2	0.14	0.18	0.26
15	6.2	0.12	0.16	0.22
10	4.1	0.10	0.13	0.18
7.5	3.1	0.09	0.11	0.16
6	2.5	0.08	0.10	0.14

Note: The indicated retention time (t_r) is the mean value of the expected retention time range.

B.4 Electrochemical detector

B.4.1 For the mobile phase, use 0.1 N acetate buffer (pH = 4.9 ± 0.1) in 15 % vol methanol/water.

Note: Prepare buffer, adjust pH, and then add methanol to bring volume composition to 15 % methanol. The buffer serves as a supporting electrolyte.

B.4.2 For a working electrode, use either glassy carbon or porous graphite.

B.4.3 For the working electrode potential, use for oxidation + 0.7 V vs. Ag/AgCl reference electrode or + 0.5 V vs. Pd reference electrode and use for reduction – 0.2 V vs. Ag/AgCl reference electrode or – 0.4 V vs. Pd reference electrode.

Note: The oxidation potential is for the oxidation of hydroquinone to quinone, and the reduction potential is for the reduction of quinone to hydroquinone.

B.4.4 The nominal flow rate for a selected internal diameter (I.D.) of a column should be as follows:

I.D. (mm)	Flow rate (mL/min)
4.6	2.0
3.9	1.4
2.0	0.4

Note: For other I.D.s, use a flow rate $(I.D.)^2 /10.6$ mL/min.

B.4.5 The retention time range for a selected length of column should be as follows:

Column length (cm)	Retention time range (min)
30	6.6 - 8.7
25	5.5 - 7.2
20	4.4 - 5.8
15	3.3 - 4.4
10	2.2 - 2.9
7.5	1.6 - 2.2
6	1.3 - 1.7

B.4.6 The average of at least three repeated measurements of the maximum output peak width at half height for various packing particle diameters and lengths of the column should be the same as the values given in the Table under B.3.6 after the values provided are multiplied by a factor of two.

ANNEX C

A PERFORMANCE TEST FOR AN HPLC SYSTEM
(Mandatory)

C.1 This test provides an overall performance test for an entire HPLC system. In addition, the test results shall be consistent with the requirements of clause 4 of this Recommendation and, therefore, can provide a test of the detector used.

Note: The results of this test may not be reliable in predicting an instrument's performance in detecting sample components required by a specific analytical method. In some cases, a test compound specific to the analytical method, therefore, should be used with this procedure for routine testing or as a means of calibrating an instrument (see 7.3.2).

C.2 This test applies to using a reversed phase HPLC system and a UV/visible spectrophotometric and/or fluorescence detector and is recommended for the overall test using a test compound of anthracene in a methanol/water solution.

C.3 Prepare a stock solution of anthracene in methanol that has a concentration of 1.5 mg/mL for testing the UV/visible spectrophotometric detector and the fluorescence detector, respectively. These values are obtained from the product of the detection limit and the linear range. If an electrochemical detector is to be tested, then the concentration and dilution factors for the test compound, hydroquinone or quinone, would be the same as that required for the UV/visible spectrophotometric detector.

Note: Anthracene may be a potential health hazard. Persons who carry out this test should be made aware of this hazard by means of a material safety data sheet and should be instructed to follow safe laboratory practices.

C.4 Prepare three additional reference solutions in 60 % methanol and 40 % water by serial dilution of the stock solution by factors of 0.1, 0.05, and 0.01 for the UV/visible spectrophotometric detector and 0.01, 0.0066, and 0.0033 for the fluorescence detector, or by factors such that the minimum concentration is not greater that 100 times that of the detection limit of the detector being tested.

Note: Acetonitrile and water may be used.

C.5 Set the flow rate appropriate for the column diameter as given in B.2.1. Adjust the mobile phase composition and/or column temperature to meet the retention time requirements given in B.2.2 for the appropriate column length. Observe the temperature requirements of 4.3 and 4.4.2. Measure the peak width at half height. If the peak width at half height does not meet the requirement of 4.4.3 or 4.4.4, replace the column before continuing with the test. (See also B.3.6 and E.3.3.)

C.6 Inject 20 µL of each diluted reference solution of C.4 in turn. Measure the absorbance of each specific reference solution at least four times and the noise level.

C.7 Record retention times, peak areas, peak heights, noise levels, and the peak widths at half height. Calculate the signal to noise ratio by dividing the peak height by the noise level. Calculate the relative standard deviation for the peak areas and retention times.

C.8 The relative standard deviation of the retention times for each diluted sample shall be less than 3 %, and the corresponding relative standard deviation of the peak areas or heights of the output signals should be less than 5 %. The maximum measured peak widths at half height shall meet the requirements specified in 4.4.3 or 4.4.4.

C.9 Plot the average of the peak area or peak height obtained in C.7 versus the amount of test compound injected in nanograms. Calculate the slope of the curve which fits the data to a least-squares-linear curve. Draw a line on this graph equal to three times the noise level obtained in C.7. The detection limit is the intersection of these two lines and shall meet the requirements of 4.4. The result of the linear least-squares fit of the data for four measurements at each of the three concentrations shall have a correlation coefficient (r^2) of 0.98 or better.

Note: The correlation coefficient (r^2) can be calculated from the slope (s) and the standard deviation of the concentration (σ_x) and the detector output signal (σ_y) as follows:

$$r^2 = \left(s \times \frac{\sigma_x}{\sigma_y} \right)^2$$

where:

$$s = \frac{\Sigma x_i^2 \, \Sigma y_i \; - \; \Sigma x_i \, \Sigma \, (x_i \, y_i)}{n \, \Sigma x_i^2 \; - \; (\Sigma x_i)^2}$$

$$\sigma_x = \left[\frac{\Sigma \, (x_i \; - \; \overline{x})^2}{n - 1} \right]^{1/2}$$

$$\sigma_y = \left[\frac{\Sigma \, (y_i \; - \; \overline{y})^2}{n - 1} \right]^{1/2}$$

$\overline{x}$ and $\overline{y}$ = mean values
n = number of data values

C.10 A direct measurement of the detection limit is given by an injection of 20 µL of the stock solution diluted by a factor of 10^{-4} for the UV/visible spectrophotometric detector and by a factor of 0.33×10^{-4} for the fluorescence detector.

C.11 Make four repeated injections of 20 µL of the stock solution of anthracene. The ratio of the average value of these results to the detection limit is the linear range and shall meet the requirements of 4.4 to within ± 5 %.

C.12 The sensitivity of the instrument is the slope of the curve obtained in C.9, or alternatively, it may be calculated according to the equation in 2.12.

ANNEX D

TEST REPORT FORMAT

Note: This Annex is informative with regard to implementation of this Recommendation in national regulations; however, use of the test report format is mandatory for the application of the Recommendation within the OIML Certificate System.

A test report intended for use in the OIML Certificate System or for other purposes shall include the following information.

D.1 The name and address of the testing laboratory(ies)

D.2 A reference (number and year of edition) to this Recommendation

D.3 A unique identification of the pattern to which this test report applies (e.g., the common trade names, model, and a brief description including drawings, diagrams, and inscriptions)

D.4 The name(s) and address(es) of the manufacturer and of the applicant if other than the manufacturer

D.5 An identification of the instrument sample(s) tested:

- model and serial number:
- date of manufacture:
- requirements for voltage:

                    frequency:

                    current:

D.6 The date of the beginning of testing:

D.7 The location at which tests were performed if other than the address provided in D.1:

D.8 The results of the review of the operating manual:

acceptable:

deficiencies:

comments:

D.9 A summary of the results of tests carried out according to 7.1.5 and the conditions specified in this Recommendation

D.9.1 Conditions and configuration

- Ambient temperature: and humidity:
- Mobile phase:

- Column: – particle diameter:
  – length:
  – inside diameter:
  – stationary phase:
  – solid support:

- Mobile phase composition achieved:
  – premixed: or
  – solvent blending by the instrument:

- Mobile phase   % by volume H$_2$O %:
  % by volume

- Flow rate: and system pressure:

- Type of detector(s) used:
  – U.V. visible:
  – fluorescence:
  – electrochemical:

- Temperature control: yes: no:

- Detector temperature: ±

- Description of the major features of the data handling system and control settings:

Comments:

D.9.2 Visual Inspection

- Pressure monitoring: yes: no:

- Pressure limit switch: yes: no:

- Guard column or filter (mesh size   ) after the pump

- System plumbing has no leaks: yes: no:
  Note: If the system is leaking, stop the test immediately until all leaks are eliminated.

Comments:

D.9.3 Test Results

- Test compound injected: volume:

Detector type	Detection limit	Linear range	Sensitivity
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Note: The above table should be completed for each detector tested.

- Attach graphical results for determining detection limit and sensitivity according to C.9.

Comments:

D.10 A brief statement of the conclusions as to whether the instrument sample(s) tested met the requirements of this Recommendation.

D.11 The signature of the responsible person(s), the date signed, and a unique report number.

ANNEX E

PROCEDURE FOR CHECKING THE OPERATIONAL READINESS
OF AN HPLC SYSTEM

E.1 This test provides a procedure for the conditioning and checking of an HPLC system before operation for an analysis (see also reference [7]).

E.2 The purpose is to provide a routine overall HPLC system check.

E.3 Start-up procedure

E.3.1 Turn on power to all HPLC-system modules, pump(s), detector(s), column oven, and recorder, or integrator.

Note: The time and date should also be set on microprocessor based systems.

E.3.2 Fill all solvent reservoirs with the appropriate degassed mobile phase solvents.

E.3.3 Flush and degas all plumbing lines prior to pumping mobile phase through column at a flow rate of 1 mL/min.

E.4 Checking procedure

E.4.1 Measure the stability of the flow rate by collecting the delivere mobile phase in a graduated cylinder over at least three successive ten minute periods (see 4.1.2).

E.4.2 Check system pressure and check the pressure fluctuations of the solvent delivery system after the column is conditioned with the mobile phase. Pressure fluctuations should be less than ± 0.2 MPa for 10 MPa head pressure.

E.4.3 Check all plumbing for solvent leaks.

E.4.4 Check the detector stability by observing the digital display or recorder and/or integrator response.

Note: This stability should be within ± 1 % of the maximum full scale attenuation.

E.4.5 Confirm that all temperature controlled components have reached their nominal instrument settings.

E.5 Conclusion

If all conditions under E.4 are acceptable, the HPLC system can be considered ready to perform a measurement.