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**General rules for high performance
liquid chromatography**

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Foreword

This translation has been made based on the original Japanese Industrial Standard revised by the Minister of Economy, Trade and Industry, through deliberations at the Japanese Industrial Standards Committee as the result of proposal for revision of Japanese Industrial Standard submitted by Japan Analytical Instruments Manufacturers' Association (JAIMA)/Japanese Standards Association (JSA) with the draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law applicable to the case of revision by the provision of Article 14.

Consequently **JIS K 0124:2002** is replaced with this Standard.

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General rules for high performance liquid chromatography

1 Scope

This Japanese Industrial Standard specifies general rules applicable to the cases when using high performance liquid chromatography for the qualitative/quantitative analysis of analyte and when performing aliquot with the purpose of purification for analysis.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 0050 *General rules for chemical analysis*

JIS K 0127 *General rules for ion chromatographic analysis*

JIS K 0211 *Technical terms for analytical chemistry (General part)*

JIS K 0214 *Technical terms for analytical chemistry (Chromatography part)*

JIS K 0215 *Technical terms for analytical chemistry (Analytical instrument part)*

3 Terms and definitions

For the purposes of this Standard, the terms and definitions given in **JIS K 0050**, **JIS K 0127**, **JIS K 0211**, **JIS K 0214** and **JIS K 0215**, as well as the following apply.

3.1 high performance liquid chromatography

method to separate and detect analyte sophisticatedly by using the difference of interaction (adsorption, partition, ion exchange, size exclusion, etc.) between stationary phase and liquid mobile phase which is passed through a column using a pump, etc.

3.2 high performance liquid chromatograph

apparatus by which high performance liquid chromatography is carried out

3.3 analyte

target component to be analyzed existing in sample or sample solution

3.4 sample solvent

a solvent used for dissolving sample so as to inject the sample into column

3.5 test sample solution

sample solution itself or that given any pretreatment, for measurement

3.6 stationary phase

the phase, one of the elements associated with separation in the field of liquid chromatography, which is in equilibrium with mobile phase and interacts with analytes

3.7 mobile phase

the phase, one of the elements associated with separation in the field of liquid chromatography, which is the liquid running in contact with and is in equilibrium with stationary phase, and causes to repeat interactions between the stationary phase and the analyte while moving and interacting with the analyte

3.8 eluent, eluant

liquid which is used as a mobile phase for developing and eluting the analyte retained in a column

3.9 eluent reservoir, eluant reservoir

reservoir to store eluent

3.10 pump

device to supply eluent (mobile phase)

3.11 sample injector

device to inject the test sample solution into the column of a high performance liquid chromatograph

3.12 automatic sample injector, auto sampler

device to automatically inject the test sample solution into the column of a high performance liquid chromatograph in succession

3.13 column

the tube, for the separation of analyte, either chromatographic tube in which column packing has been packed, or capillary tube, etc. of which inside wall surface retains substance to be a stationary phase

3.14 column compartment

a compartment in which column are kept for the purpose of controlling temperature

3.15 detector

device to detect characteristic values (such as absorbance and refractive index) of analyte separated in the column and to convert them into electric signals such as voltage

3.16 column packing

mainly granular stationary phase to be packed inside a chromatographic tube, grain on which stationary phase has been retained or stationary phase of monolith

3.17 monolith

a stationary phase having the skeletal structure of totally porous silica gel or polymer made to be the base material

It includes that of rod type or that synthesized in capillary of fused silica, etc.

3.18 chromatographic tube

cylindrical tube in which column packing is packed and whose both ends are provided with filters and the like

3.19 eluate, effluent

liquid flowing out from the column after development by the eluent (mobile phase)

3.20 theoretical plate number

one of the indexes that indicates column efficiency by the total plate number in the entire column where a column is regarded theoretically as a series of discontinuous plates of small section

There are methods such as the tangent method, the peak width at half height method, the area height method depending on the method obtaining the peak width. They are obtained according to the following formulae.

$$N=16 \times \left(\frac{t_R}{W} \right)^2 \dots\dots\dots \text{Tangent method}$$

$$N=5.54 \times \left(\frac{t_R}{W_{0.5h}} \right)^2 \dots\dots\dots \text{Peak width at half height method}$$

$$N=2\pi \times \left(\frac{t_R H}{A} \right)^2 \dots\dots\dots \text{Area height method}$$

where, N : the theoretical plate number

t_R : the retention time

W : the peak width

$W_{0.5h}$: the peak width at half height

A : the peak area

H : the peak height

And, for t_R and W , t_R and $W_{0.5h}$, the same unit shall be used.

3.21 resolution

a measure to indicate the degree in which the peak of target component is separated from the neighbouring peak

It is obtained according to the following formula.

$$R=1.18 \times \left(\frac{t_{R2} - t_{R1}}{W_{0.5h1} + W_{0.5h2}} \right)$$

where, R : the resolution

t_{R1} , t_{R2} : the retention time of each neighbouring peak
($t_{R1} < t_{R2}$)

$W_{0.5h1}$, $W_{0.5h2}$: the peak width at the centre of height of each neighbouring peak

And, for t_{R1} , t_{R2} , $W_{0.5h1}$ and $W_{0.5h2}$ the same unit shall be used.

3.22 symmetry factor

a factor that indicates the degree of symmetry of peak on chromatogram

It is obtained by the following formula.

$$S = W_{0.05h} / 2f$$

where, S : symmetry factor

$W_{0.05h}$: peak width at 1/20 height of peak height measured from baseline of peak

f : the distance of leading side of peak when the peak width $W_{0.05h}$ is bisected by a perpendicular drawn from the peak top toward horizontal axis of recording paper

The same unit shall be used for $W_{0.05h}$ and f .

3.23 precolumn derivatization, precolumn labeling

procedures to derivatize an analyte prior to introduction of it into column by reaction with derivatizing agents

3.24 postcolumn derivatization, postcolumn labeling

procedures to derivatize an analyte separated in column by adding derivatizing agents to the eluate

3.25 isocratic elution

the procedure to develop and elute an analyte by using a single constituent eluent (mobile phase)

3.26 gradient elution

the procedure to develop and elute analyte while the constituents of mobile phase (eluent) are being changed

3.27 reference solution

the solution whose characteristic values such as concentration or molecular weight (formula weight), have been known and confirmed to the extent necessary and sufficient to be used for high performance liquid chromatography as the standard for analysis

3.28 separation mode

a mode classified based on the mode of action mainly controlling separation in a high performance liquid chromatography

3.29 base line

the signal that is recorded when only the eluent passes the detector without any analyte peaks in the chromatogram

3.30 diluted reference solution

the reference solution that has been diluted to the specified concentration

3.31 mixed reference solution

the solution prepared by mixing plural reference solutions or diluted reference solutions with each component adjusted to predetermined concentration

3.32 internal standard

substance used as a standard in the internal standard method

3.33 molecular weight distribution

diagram that represents quantity of respective molecular species as a function of each molecular weight of a compound such as normal polymer whose molecular weight has polydispersity

3.34 preparative liquid chromatography

liquid chromatography that is intended to collect fractions containing analytes

3.35 fractionation

operation to take out analytes that have been separated and eluted in the column

3.36 fraction

solution that has been divided by fractionation operation

3.37 dead volume

the holding capacity for components that are inactive to the column

It is related with free gas space (void volume), sample injector, piping, and detector's effective volume. It is also called a hold-up volume.

3.38 method validation

various tests to demonstrate that the analysis method is suitable for the purpose of analysis by using high performance liquid chromatography

It is also called validation.

4 Overview of high performance liquid chromatography

Liquid chromatography (LC) is one of methods for physical, chemical separation analysis having the following features. It uses liquid as mobile phase. There occurs the difference of interaction of each analyte between stationary phase and mobile phase of column. The difference is used to separate mixture. The analysis method where the mobile phase is pressurized with a pump and conveyed to achieve high performance separation in a short time is called high performance liquid chromatography (HPLC).

LC can be applied to measure such non-volatile or thermally "unstable compounds that are difficult to measure by gas chromatography. LC is less capable of separation than gas chromatography, because the dispersion speed of LC is slower. However, LC is featured with excellent quantification and easy aliquot of separated compounds though separation capability is not so high as compared with a gas chromatography. LC is applied not only to general organic substances but also to ionic compounds, natural substances, polymer compounds, and other wide fields.

HPLC can realize various separation mechanisms by combination of stationary phase and mobile phase used. Table 1 shows the separation mode, characteristics, typical column packing and use of HPLC.

Table 1 Separation mode, characteristics, typical column packing, and use of HPLC

Separation mode	Characteristics	Typical column packing	Use
Partition chromatography (including reverse phase partition chromatography, forward phase partition chromatography, and reverse phase ion pair chromatography)	• Separation based on partition balance between stationary phase and mobile phase. • Combination of various column packing and various mobile phases permits application to extensive objects.	Silica gel, organic silica gel, porous polymer, etc. with octadecyl radical, amino radical, etc. introduced.	• Separation of substances ranging from low molecular weight to high molecular weight. • Separation of homologue.
Adsorption chromatography	• Separation based on adsorption balance of solute by inorganic oxide stationary phase. • Non-polar organic solvent is used as mobile phase.	Silica gel, alumina, titania, carbon, etc.	• Separation of polar substances and isomer.
Hydrophilic interaction chromatography (HILIC)	• Separation based on hydrophilic interaction • High polarity of stationary phase is used. For the mobile phase, an organic solvent capable of mixing in water is used.	High polarity of silica gel, chemical bonding type silica gel with amino radical, diol radical, zwitterionic group, etc. introduced, porous polymer containing hydrophilic radical, etc.	• Hydrophilic compound such as sugar, amino acid
Ion exchange chromatography (including ion-exclusion chromatography and ion chromatography)	• Separation is based on electrostatic interaction between ion exchanger and ionic solute. • Applicable to extensive target.	Silica gel, synthetic polymer with ion exchanging radical such as carboxy methyl radical, diethylaminomethyl radical.	• Separation analysis of ionic substances, desalination, salt exchange
Size exclusion chromatography (gel filtration chromatography, molecular sieving chromatography)	• Separation based on polymer packing network or porous molecular sieving. • Treatment conditions are mild. • Hydrophilic polymer packing and synthetic polymer packing are different in purpose of use.	Dextran gel, agarose gel, polystyrene gel, polymethacrylate gel, silica gel, etc.	• Separation and purification of protein, enzyme, etc., desalination (hydrophilic polymer packing). • Molecular weight fractionation of synthetic polymer (synthetic polymer packing).
Affinity chromatography	• Separation based on molecular identification derived from biological origin. • Selectivity is very high.	Carrier in conjunction with enzyme, peptide, saccharides, etc.	• Condensation, separation and purification of physiologically active substances.

Basic configuration of HPLC is shown in figure 1. HPLC consists of the following components: a pumping system for mobile phase (a tube pump), a sample injection part (a sample injector), a column and column compartment part, a detection part (a detector) and a data processing part (a data processor and a recorder). The flow rate of mobile phase is set by the pump to be sent to the sample injection part and to the column constantly. The pump is required to have the flow rate of usually range of 0.01 ml/min to 10 ml/min and the discharge pressure of about 40 MPa. A reciprocating plunger pump as shown in figure 2 is mainly used. When the gradient eluent method is used, one to four units of pumps or a gradient device is used.

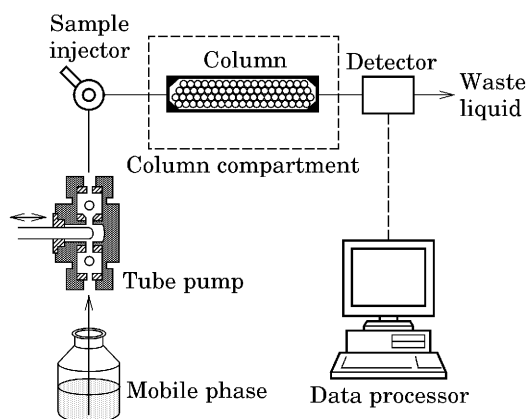


Figure 1 Basic configuration of liquid chromatograph

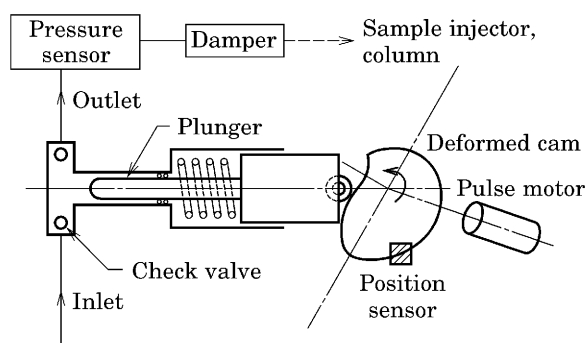


Figure 2 Basic configuration of reciprocating plunger pump (example)

The sample solution is measured with a microsyringe, etc. The specified volume of solution is injected into the column by a sample injector. Sometimes an autosampler that injects many samples sequentially and automatically is used.

The column packing used for HPLC have relatively uniform particle size of 1 μm to 20 μm in diameter and they are packed in the stainless steel or plastic chromatographic tube with inside diameter of 1 mm to 12 mm and maximum length of several dozen centimetres. The particle diameter of column packing and the size of chromatographic tube are different depending upon their purposes of use. The monolithic type column being a continuum formed to a rod shape, the micro column under 1 mm in inside diameter, the semi aliquot column of not less than 12 mm in inside diameter are also used. The performance of column is indicated by the theoretical plate number, resolution, symmetry factor, etc. Analytes separated by column are detected by the detector based on their physical, optical, electrical, chemical and other characteristics. A typical detector for HPLC is an ultraviolet-visible spectrophotometric detector. Usually, the detector is provided with a flow cell of 4 μl to 20 μl , and it measures absorbance (absorption intensity) of the separated analyte at the specified wavelength. Other detectors include fluorescence detector, differential refractometer detector, electrochemical detector, conductivity detector, mass spectrometer, evaporation light scattering detector, charged aerosol detector, ICP emission spectrophotometer, ICP mass spectrometer, etc. The typical detectors used for HPLC and their characteristics are shown in table 2. When selective detection or high sensitivity detection of $\mu\text{g/L}$ or less is required, different detectors shall be used depending upon the characteristics of the analytes.

When analytes cannot be directly detected or when high sensitivity detection is impossible, precolumn derivatization or post column derivatization is used. The former is derivatization performed before analytes are injected into column. The latter is derivatization performed after analytes are separated in the column and the derivatization reagents are mixed with eluates.

The response of each analyte obtained by the detector is converted into electric signal and sent to a data processor. A chromatogram that records the response from detector against the time axis, as shown in figure 3 is obtained. The data processor calculates various parameters based on this chromatogram, and performs qualitative/quantitative analysis of the analytes. As to the qualitative analysis of each separated analyte, the retention value (retention time, etc.) is compared with that of chromatogram of the reference solution obtained under the same condition. When a mass spectrometer or a photodiode array type spectrophotometric detector is used, the spectrum is compared with that of reference solution and qualitative analysis is performed. As to the quantitative analysis, the peak area value or peak height value is obtained from the chromatogram. It is applied to the calibration curve that has been prepared by reference solution containing analytes beforehand and determination is performed.

On the other hand, it is possible to perform aliquot/purification of separated analytes, by sampling the eluate that corresponds to the peak of chromatogram.

For the purpose of improving the productivity of analysis, the ultrahigh-speed analysis which shortens the analysis time much more than before is carried out in some case. The ultrahigh-speed analysis has such advantage as it can analyze samples in less time and it needs less time to consider the analytical condition, and it can be realized by the methods such as feeding mobile phase under high pressure and using the exclusive column.

Table 2 Typical detectors used for HPLC and their characteristics

Detector	Minimum detection sensitivity g	Selectivity	Influence of temperature	Influence of flow speed	Gradient elution
Ultraviolet-visible absorption detector	10^{-11}	Yes (absorbance substance)	Small	No	Possible
Fluorescence detector	10^{-13}	Yes (fluorescent substance)	Yes	No	Possible
Differential refractometer detector	10^{-7}	No	Yes	No	Impossible
Electrochemical detector	10^{-12}	Yes (oxidation-reduction substance)	Yes	Yes	Possible
Conductivity detector	10^{-10}	Yes (ion)	Yes	Yes	Difficult
Mass spectrometer	10^{-14}	No	Yes	Yes	Possible
Evaporation light scattering detector	10^{-8}	No	No	No	Possible
Charged aerosol detector	10^{-9}	No	No	No	Possible
ICP emission spectrophotometer	10^{-11}	Yes	No	No	Possible
ICP mass spectrometer	10^{-14}	Yes	No	No	Possible

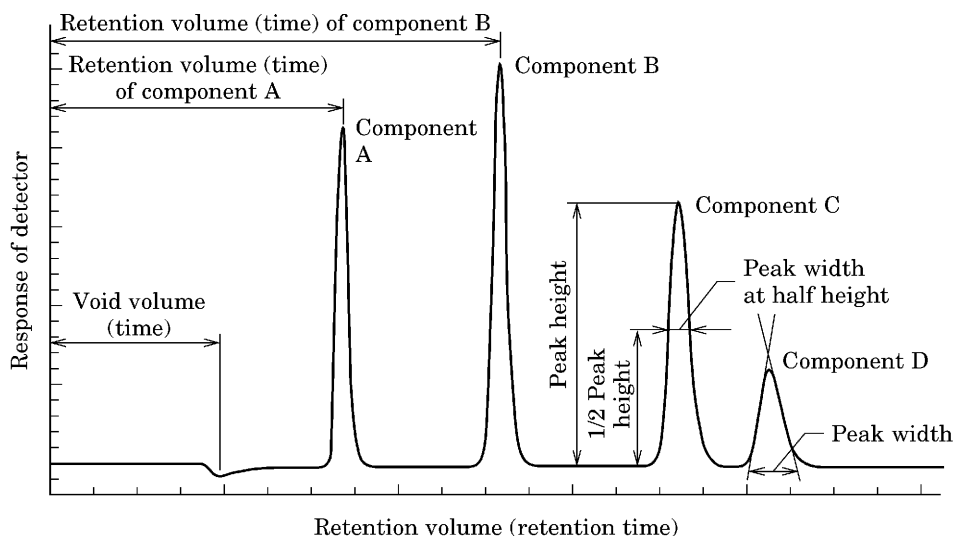


Figure 3 Chromatogram and name of each parameter

5 Instrument

5.1 Configuration of instruments

The high performance liquid chromatograph consists of the a pumping system for mobile phase, a sample injection part, a separation part, a detection part and a data processing part, etc. and an example of the basic constitution is shown in figure 4. The sample, reagent and carrier constitute the continuous flowing system in the capillary.

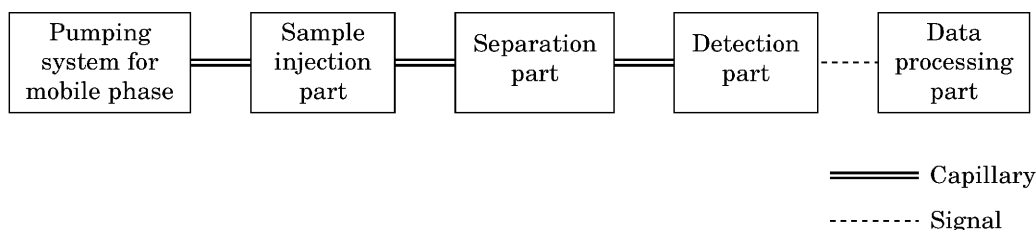


Figure 4 High performance liquid chromatograph (example)

5.2 Pumping system for mobile phase

The apparatus part which sends the eluent (mobile phase) to the column, mainly consisting of the following parts.

- Eluent reservoir** The eluent reservoir shall be made of materials that do not affect chemical composition of the eluent (mobile phase) and shall prevent pollution from environment as well as evaporation/deterioration of the mobile phase while in use.
- Deaerator** It is used to continuously remove air dissolved in the eluent (mobile phase), to prevent bubble trouble that be caused by the change of temperature and pressure within the equipment, and to ensure stable flow rate and background.
- Tube pump** The pump for sending the eluent (mobile phase) set up with accurate and precise flow rate to the column, preferably meeting the following requirements:

- 1) capable of setting a wide range of flow rate;
 - 2) capable of setting highly precise flow rate;
 - 3) to have low pulsating flow;
 - 4) capable of obtaining stable discharge against a wide range of feeding pressure;
 - 5) of which materials contacting with liquid are excellent in chemical resistance
 - 6) to facilitate exchange of mobile phase.
- d) **Gradient elution part** It consists of a control part where plural eluents (mobile phases) to be used change temporally their composition and a mixing part where the mixed solution is made to be homogeneous. The setting range of mixing ratio should be wide, and the concentration should be accurate and precise.

5.3 Sample injection part

There are manual sample injectors to inject manually by using a micro syringe, etc. and the automatic sample injectors to inject automatically multiple test sample solutions in succession. In any case, the materials and construction should be such that the injected samples are less remained and not cause adsorption, etc. to ensure reproducible introduction of the fixed quantity of test sample solution into the system.

When using a manual injector, there are measuring methods such as to measure the required quantity of sample solution by a micro syringe and to measure by a definite capacity of sample loop attached to the device. When using an auto sampler, there are various systems. According to the classification by measuring method, there are such methods as to measure by a sample loop of definite capacity and to measure by a syringe or the suction volume of pump. According to the classification by introducing system to column, there are such systems as only the sample loop is connected to the column flow passage, and as the needle, in addition to the sample loop, is also connected to the column passage directly. If an automatic sample injector is used to inject multiple test sample solutions automatically, such basic functions as reproducibility of injection volume, accuracy, low remained amount and low minimal sample amount should be provided in the specification. Furthermore, such functions suitable for the use as cooling to decrease decomposition, volatilization of the analytes should be added.

An automatic sampler which incorporates the function of sample pretreatment or precolumn derivatization may be used.

5.4 Separation part

The separation part shall be as follows.

- a) **Column** According to the analytical purposes, use the column specified in clause 7. Take care to minimize dead volume in the piping with the inlet channel from the sample injection part and with the outlet channel to the detector as far as possible in order to prevent sample bands from spreading.

A guard column may be used to prevent performance deterioration of the analysis column.

- b) **Column compartment** Use a column compartment to keep the separation of the analytes in samples constant and to ensure determination precision. The column compartment should be equipped with temperature control function. Set the temperature so as to optimize column performance, to enhance separation selectivity, and to prevent the column temperature from being affected by changes in environmental temperature, etc.

5.5 Detection part

The detection part introduces the eluate from the column to the micro-flow cells, and detects concentration or volume of analytes based on various principles, by taking the mobile phase as a background, and shall be as follows.

- a) **Ultraviolet-visible absorption detector** It detects absorbance of analytes absorbed in the ultraviolet and visible regions. The absorption wavelength is set based on the absorption characteristics of analytes. Such an absorptiometric detector that sends dispersed light to a flow cell is commonly used. In the case of a photo diode array detector, qualitative analysis of analytes and confirmation of the purity of peak components are performed based on chromatogram for each wavelength and each retention time obtained by measurement of absorbance change at each wavelength of light dispersed after the flow cell.
- b) **Fluorescent detector** It detects the intensity of fluorescent from the analyte which generates fluorescence of specified wavelength by activation with the exciting light. In order to set the optimal wavelength and to confirm peak component qualitatively, some detectors can perform activation and collection of fluorescent spectra by activation and wavelength scan of fluorescence generated.
- c) **Differential refractometer detector** It detects the change of refractive index of the mobile phase solution due to elution of analytes as a difference of refractive index.
- d) **Electrochemical detector** It detects currents produced by oxidation and reduction of the analyte at the working electrodes to which a specified voltage is applied.
- e) **Conductivity detector** It detects difference of conductivity between the eluent (mobile phase) and the analyte.
- f) **Mass spectrometer** It detects the analytes that have been eluted from the column and ionized according to their m/z (mass-to-charge ratio).
- g) **Evaporation light scattering detector** It detects the intensity of scattering light generated by the light irradiation to spherical fine particles resulted from evaporation of the eluent (mobile phase).
- h) **Charged aerosol detector** It detects the charge amount of spherical fine particles resulted from evaporation of the eluent (mobile phase) then positively charged by collision with nitrogen ion which has been positively charged by corona electrode.
- i) **ICP emission spectrophotometer** It detects the emission intensity of atomic spectral line obtained by vaporization and excitation of analyte (element) introduced in the high temperature argon plasma generated by using high frequency.

- j) **ICP mass spectrometer** It detects ion generated by ionization of the analyte (element) by using the high frequency inductively coupled plasma and then introduced into mass spectrometer to be detected corresponding to m/z (mass-to-charge ratio) of the analyte (element).
- k) **Other kinds of detectors** Other kinds of detectors include the following.
- 1) Infrared spectrophotometer
 - 2) Optical rotation detector, circular dichroism detector
 - 3) Flame ionization detector
 - 4) Radiation detector
 - 5) Permittivity detector
 - 6) Chemical luminescence detector
 - 7) Atomic absorption spectrometer
 - 8) Thermal detector
 - 9) Light scatter detector
 - 10) Viscosity detector
 - 11) Ion electrode
 - 12) Ultrasonic detector
 - 13) Nuclear magnetic resonance apparatus

5.6 Data processor

Data processor shall display the chromatogram and the quantitative results calculated by the function of preparing the calibration curve and wave form processing needed for the determination from retention time, peak height, peak area, etc. Some data processors have spectral analysis function.

5.7 Accessories

According to the analytical purposes, the following accessories shall be provided, if necessary.

- a) Computer (for workstation, for data processing/control)
- b) Path switching valve (column switching, recycle, back flush, etc.)
- c) Fraction collector
- d) Post column reactor
- e) Eluent recovery apparatus

6 Water, reagent and solvent

The water, reagent and solvent shall be as follows.

- a) **Water** The water used for this Standard is the refined water by the combination of such methods as the reverse osmosis, distillation, ion exchange, ultraviolet

irradiation and filtration and the quality of water shall not disturb analysis. The specific resistance value, total organic compounds (TOC), absorbance, etc. are evaluated as the indication of the quality of water. It is deteriorated by suffering the pollution from the container and environment soon after use, therefore the container of material not influencing on analysis shall be selected, and used after washed by an appropriated method.

- b) **Reagent and solvent** Qualities of reagent and solvent shall be such that not influence on the analysis except the original property. There can be cases where many grades exist, therefore that suitable for the purpose shall be selected. After the seal is opened, that is deteriorated by suffering the pollution from environment, therefore, that shall be used as soon as possible. Also, the elapse of time after manufactured results in the change of quality in some cases, therefore the new one shall be used as much as possible.

7 Column and column packing

7.1 Column

The column shall be as follows.

- a) **Marking of column type** Columns are available in various kinds, depending on combination. The specification of column shall be indicated by material, length, and inside diameter of chromatographic tube, as well as name of column packing.
- b) **Material of chromatographic tube** The tube shall provide sufficient strength, the inner surface of which shall be smooth, and the material used for which shall be inactive against the eluent and analyte, such as stainless steel, polyether ether ketone (PEEK), glass lining stainless steel, PEEK lining stainless steel and fused silica tube.
- c) **Classification of column** Columns are available in various sizes, depending on type, quantity, and characteristics of analysis sample, as well as analytical conditions. They are classified as follows by the inside diameter.
- 1) Semi-aliquot column: 10 mm or over to and excluding 50 mm in inside diameter
 - 2) Column for general use: 3 mm or over to and excluding 10 mm in inside diameter
 - 3) Semi-micro column: 1 mm or over to and excluding 3 mm in inside diameter
 - 4) Micro column: Under 1 mm in inside diameter

7.2 Column packing

For the column packing, the particle of which the particle size is comparatively uniform and about 1 μm to 20 μm and the monolith type one being a continuum, etc. are used, and is stable physically and chemically under the working conditions.

The materials of the column packing include inorganic compound such as silica gel, organic silica gel having the silica gel skeleton with methyl radical or ethylene radical incorporated, polymer compound such as polystyrene gel, the substance of which the surface is bonded chemically with organic molecule on the above materials as the matrix.

7.3 Separation mode and column packing

HPLC is available in various separation modes, depending on combination of column packing and eluent. According to physical/chemical characteristics of analyte, an appropriate selection mode needs to be selected.

7.4 Column performance evaluation

The column performance is sometimes evaluated in terms of the peak shape, theoretical plate number, symmetry factor, column life, resolution, adsorption of basic compounds, selectivity, maximum sample load, etc.

When the column performance is evaluated, it shall be conducted under the condition where it is less affected by environment and apparatus based on purpose, and reproducibility can be obtained easily.

8 Operation

8.1 Preparation for sample

High performance liquid chromatograph analysis requires selection of sampling method suitable to a sample according to its analysis purpose before use. Depending on the chemical form and analysis purpose, the collected sample may be either injected directly into a high performance liquid chromatograph, or subjected to pretreatment such as analyte extraction, derivatization, concentration, etc.

8.1.1 Sampling

Sampling shall be performed using the best method so as to suit the analysis purpose, sample conditions and test items, and to represent the sample population or to clarify the difference of characteristics of each sample. There are cases where the quantity varies according to the sampling season and time, and where the individual difference of analyte and the internal heterogeneity are high depending on the object substance. Select the condition of sampling object substance, site, time and method according to the cases such as aiming to obtain overall mean values which are independent of time, space and individual and aiming to observe temporal variation of the sample, the difference due to the sampling place, and the characteristic of sample. If an individual standard is available, comply with it. To prevent deterioration due to external factors, ageing, etc., perform pretreatment and sample injection to the high performance liquid chromatograph as soon as possible. Some samples can undergo biological, chemical or physical change in the course of storage. In the case of a sample of which denaturation is inevitable, measure it immediately after sampling.

8.1.2 Sample storage

When required to store the sample, confirm beforehand that there is no problem with respect to the deterioration of sample by the method of storage and transportation. Take care not to set conditions such as the amount of sample or concentration of analyte handled in the preliminary experiment is widely different from the level of this measurement, because the conditions to be set become not effective. If deterioration is a concern, take measures¹⁾ to prevent it. Use the container and instrument for sampling and storing of which materials are free of such a component as adsorbs the sample or leaches into the sample. Label the storage container with necessary precautions. If