



JAPANESE
INDUSTRIAL
STANDARD

Translated and Published by
Japanese Standards Association

JIS K 0135 : 2003

(JBA/JSA)

General rules for preparative liquid chromatography

ICS 71.040.50

Reference number : JIS K 0135 : 2003 (E)

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Foreword

This translation has been made based on the original Japanese Industrial Standard established by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee according to the proposal of establishing a Japanese Industrial Standard from Japan Bioindustry Association (JBA)/Japanese Standards Association (JSA), with a draft of Industrial Standard based on the provision of Article 12 Clause 1 of the Industrial Standardization Law.

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Date of Establishment: 2003-12-20

Date of Public Notice in Official Gazette: 2003-12-22

Investigated by: Japanese Industrial Standards Committee

Standards Board

Technical Committee on Chemical Products

JIS K 0135:2003, First English edition published in 2004-09

Translated and published by: Japanese Standards Association
4-1-24, Akasaka, Minato-ku, Tokyo, 107-8440 JAPAN

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Printed in Japan

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General rules for preparative liquid chromatography

1 Scope This Japanese Industrial Standard provides for general rules of the preparative liquid chromatography used for the fractionation of the material.

2 Normative references The following standards contain provisions which, through reference in this Standard, 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 0114 *General rules for gas chromatographic analysis*

JIS K 0124 *General rules for high performance liquid chromatography*

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 3600 *Biotechnology—Vocabulary*

JIS K 3610 *Technical terms for biological engineering*

JIS Z 8103 *Glossary of terms used in measurement*

JIS Z 8122 *Contamination control—Terminology*

3 Definitions For the main terms used in this Standard, the definitions given in **JIS K 0114**, **JIS K 0124**, **JIS K 0127**, **JIS K 0211**, **JIS K 0214**, **JIS K 3600**, **JIS K 3610**, **JIS Z 8103**, **JIS Z 8122** and the following definitions apply.

a) **General terms** The definitions of the general terms are as follows:

- 1) **preparative liquid chromatography** A separation method whereby target components in the sample solution are fractionated and collected with the chromatography using a liquid as a mobile phase.
- 2) **purification** Procedure for increasing the purity of a target component by removing the impurities contained in a target component of a sample solution.
- 3) **packing layer** Layer of column packing packed into a chromatograph tube.
- 4) **load, sample injection** Procedure for injecting a samples into a column.
- 5) **load mass, load volume** Mass or volume of the sample which is injected into a column.
- 6) **load solvent, sample solvent** Liquid for dissolving the sample.
- 7) **elution** Procedure for developing and eluting the components which are retained in a column.
- 8) **elution** Procedure for flowing out the components retained in a column by using a liquid.

- 9) **cleaning** Procedure for eluting the components retained in a column after target substance is fractionated out.
 - 10) **cleaning solvent** Liquid for eluting the components retained in a column after target substance is fractionated out.
 - 11) **regeneration** Procedure for restoring a column packing which is used as fractionation, to the initial condition.
 - 12) **reequilibration** Procedure for restoring a column which has been used for fractionation, to the initial condition.
 - 13) **fractionation** Procedure for fractionating the analytes that have been separated and eluted with the column.
 - 14) **fraction** Solution which is divided into the fractions.
 - 15) **solvent recovery** Procedure for collecting the solvent used in fractionation for recycling.
 - 16) **challenge test** Test for confirming whether the quality of a product, manufactured under the condition of assuming that the quality comes to the worst, in the main manufacturing process, is within the threshold limit value of the standard.
- b) **Terms on apparatus** Definitions of terms on apparatus shall be as follows:
- 1) **precolumn** Column set up before a main column, in order to remove the unnecessary components and then send the fraction which is contained the required component, into the main column.
 - 2) **guard column** Column set up before a main column in order to prevent the deterioration of the main column, due to impurities in the sample and the highly retained components.
 - 3) **scavenger column** Column set up before the main column, because of removing the impurities and particulate matters due to the solvent of mobile phase.
 - 4) **frit, column filter** Perforated plate for holding the column packings in a column and passing through the mobile phase.
 - 5) **distributor** Straightening vane set up on the inlet and/or the outlet of a column, whereby the sample and/or the mobile phase is loaded and fed uniformly in the direction to cross section of the column.
 - 6) **column oven** Oven for taking in the column, in order to keep the column at constant temperature.
 - 7) **column jacket** A jig which is set around the column, in order to keep the column at constant temperature.
 - 8) **empty column** Tube where a column is stored.
 - 9) **column end fitting, column terminator** Jig for introducing the solution into the column or flowing out it from the column.

- 10) **end fitting adjustable column, adjustable-terminator column** Column that is designed so as to adjust the position of the column end fitting according to its size.
 - 11) **axial compression column** Movable column where pressure is applied in the direction of the solvent flow.
 - 12) **radial compression column** Column which can be applied pressure perpendicular to the flow of the solvent in the column.
 - 13) **automatic sampler** Apparatus for loading the sample automatically into the column.
 - 14) **photodiode array detector** Apparatus where the time-varying of the absorption spectrum and the absorbance of sample component in the mobile phase is continuously measured without stopping the flow of mobile phase.
 - 15) **damper** Apparatus for decreasing the pulsating flow due to a pump.
- c) **Terms on the method** Definitions of terms on the method shall be as follows:
- 1) **single column separation system** Chromatographic system whereby the components in the sample solution are divided and collected by using one column.
 - 2) **multiple column separation system** Chromatographic system whereby the components in the sample solution are divided and collected by joining more than one column.
 - 3) **column switching** Method whereby the fractionation is carried out by switching more than one column. This is a fractionation method whereby the fraction separated with the initial column is loaded into other column and then fractionated by separation. In this case, a switching valve is employed for changing the columns, on another occasion, different separation modes are combined.
 - 4) **semi-continuous sample injection method** The loading method at time interval adjusted so that the peak of the target component in the sample loaded in the first step and the peak of another target component which is loaded in the second step may not be overlapped each other.
 - 5) **simulated moving bed chromatography** Continuous chromatography which is carried out by switching the position for an inlet or an outlet of the raw material and the eluent without moving the stationary phase.
 - 6) **isocratic elution** Method that component in the sample is developed and eluted by using the eluent of constant composition.
- d) **Terms on operation parameter** Terms on operation parameter shall be as follows:
- 1) **packing density** The value that the mass of column packings in an empty column is divided by bed volume.
 - 2) **bed volume, abbreviation: BV** Capacity of column packings in a column (value that height of packed bed is multiplied by cross-section of the column).

- 3) **solvent strength** Elution strength of the solvent when the component of the sample held in a column is eluted. The solvent that the component of the sample is eluted faster, is called "the solvent strength is stronger" than that of the solvent being slow for elution.
 - 4) **linear velocity, abbreviation: LV** Speed of mobile phase in the column. The value that the flow rate per unit time is divided by cross-section area of the column.
 - 5) **space velocity, abbreviation: SV** The value that capacity of mobile phase fed into the column at one hour is divided by the volume of column packings in a column.
 - 6) **load ratio** Ratio of the load volume to the volume of column packings which is packed in a column. Where, for the column packings which are sold under the dry condition such as silica gel system, mass of the column packings is used. And for the column packings which are sold under the wet condition such as polymer system, volume of the column packings is used. To prevent the confusion, units of load ratio (mass %, or mass/volume %) shall be written clearly.
 - 7) **purification rate** Purification mass of target substance per unit time.
 - 8) **peak broadening** Distribution width of the sample components in the effluent.
 - 9) **asymmetry factor, abbreviation: As** When the peak width on the position of 10 % peak height from the base line is divided into two by the perpendicular line taken down from the peak top to the base line, asymmetry factor means the value that the length of the peak down side is divided by the length of the peak up side ($As_{0.1}$).
 - 10) **scale up factor** Magnification for scale up.
 - 11) **quality control** Management technique which is composed of repeatability, reproducibility, coefficient of variation, abbreviation: CV, etc. (refer to **JIS Z 8103**).
- e) **Other terms** Other terms shall be as follow:
- 1) **exclusion limit** Upper limit of molecular size which can enter into the pore size of the column packings.
 - 2) **SEC Calibration curve** Related line between the elution volumes of sample components and logarithmic values of the molecular weight used in the size exclusion chromatography.
 - 3) **highly retained sample** Component which is not eluted easily by cleaning because of large retention volume.
 - 4) **ionpairing reagent** Ionic components and ionic compounds which form ion pair.
 - 5) **dead space** Part whereby the flow of mobile phase is stayed.