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Standard Practice for Liquid Chromatography Terms and Relationships[1]

This standard is issued under the fixed designation E 682; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice deals primarily with the terms and relationships used in liquid column chromatography. However, most of the terms should also apply to other kinds of liquid chromatography, notably planar chromatography such as paper or thin-layer chromatography.

NOTE 1—Although electrophoresis can also be considered a liquid chromatographic technique, it and its associated terms have not been included in this practice.

1.2 Since most of the basic terms and definitions also apply to gas chromatography, this practice uses, whenever possible, symbols identical to Practice E 355.

2. Referenced Documents

2.1 *ASTM Standards:*

D 3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships[2]

E 355 Practice for Gas Chromatography Terms and Relationships[3]

E 1151 Practice for Ion Chromatography Terms and Relationships[3]

3. Names of Techniques

NOTE 2—In the chromatographic literature one may often find the term" high-performance (or high-pressure) liquid chromatography," abbreviated as HPLC. This term was introduced to distinguish the present-day column chromatographic techniques employing high inlet pressures and columns containing small diameter packing from the classical methods. The utilization of this term or any derivative term (for example, HPLSC for high-performance liquid-solid chromatography) is not recommended.

Similarly, the use of the term "high-performance thin-layer chromatography," abbreviated as HPTLC, describing newer variations of thin-layer chromatography, is also not recommended.

3.1 *Liquid Chromatography*, abbreviated as LC, comprises all chromatographic methods in which the mobile phase is liquid under the conditions of analysis. The stationary phase may be a solid or a liquid supported by or chemically bonded to a solid.

3.2 The stationary phase may be present on or as a plane (*Planar Chromatography*), or contained in a cylindrical tube (*Column Chromatography*).

3.3 Separation is achieved by differences in the distribution of the components of a sample between the mobile and stationary phases, causing them to move along the plane surface or through the column at different rates (differential migration).

3.3.1 In *Planar Chromatography,* the differential migration process will cause the sample components to separate as a series of spots behind the mobile phase front.

3.3.2 In *Column Chromatography,* the differential migration process will cause the sample components to elute from the column at different times.

3.3.3 In *Dry-Column Chromatography,* mobile phase flow is stopped as soon as the mobile phase has reached the end of the column of *dry* medium. This column can be glass or a rigid or flexible solvent compatible plastic. Solute visualization and recovery are from the extruded or sliced column packing.

3.3.4 In *Flash Chromatography*, mobile phase flow is continued after the mobile phase has reached the end of the column of *dry* medium until elution of the desired components is achieved. Often low pressures, compatible with the materials of construction of the column, are applied to the top of the column to speed up the elution.

3.4 The basic process of selective distribution during the chromatographic process can vary depending on the type of stationary phase and the nature of the mobile phase.

3.4.1 In *Liquid-Liquid Chromatography,* abbreviated LLC, the stationary phase is a liquid and the separation is based on selective partitioning between the mobile and stationary liquid phases.

3.4.2 In *Liquid-Solid Chromatography,* abbreviated as LSC, the stationary phase is an interactive solid. Depending on the type of the solid, separation may be based on selective adsorption on an inorganic substrate such as silica gel, or an organic gel. In this definition, *Ion-Exchange Chromatography* is considered to be a special case of LSC in which the interactive solid has ionic sites and separation is due to ionic interaction.

3.4.2.1 In this definition, *Ion Exchange Chromatography* is considered to be a special case of LSC in which the interactive solid has permanently bonded ionic sites and separation is due

[1] This practice is under the jurisdiction of ASTM Committee E19 on Chromatography and is the direct responsibility of Subcommittee E19.01 on Definitions, Nomenclature, and Standard Data.

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[2] *Annual Book of ASTM Standards*, Vol 08.02.

[3] *Annual Book of ASTM Standards*, Vol 14.02.

to electrostatic interaction.

3.4.2.2 In this definition, *Ion Pair Chromatography* is considered to be a special case of LSC in which ionic counterions are added to the mobile phase to effect the separation of ionic solutes. In this technique both electrostatic and adsorptive forces are involved in the separation.

NOTE 3—Other terminology for this technique include, but are not limited to, extraction chromatography, paired ion chromatography, soap chromatography, ion pair extraction chromatography, ion pair partition chromatography, and ion interaction chromatography, but utilization of these terms is not recommended.

3.4.2.3 In this definition, *Affinity Chromatography* is considered to be a special case of LSC in which special ligands are bonded to a stationary phase so that bio-specific interactions (for example, antibody/antigen, enzyme/substrate) may be invoked to effect the separation.

3.4.2.4 In this definition, *Ion Chromatography* is considered to be a special application of LSC in which the ion exchange mechanism is still effecting the separation. Special columns or devices, after the separating column, may be needed to remove higher concentrations of inorganic ions which might otherwise interfere with the detectability using conductivity. See Practice E 1151 for further details of nomenclature for this technique.

3.4.2.5 In this definition, *Hydrophobic Interaction Chromatography*, is considered to be a special application of LSC in which the separation is based upon interaction of the hydrophobic moieties of the solutes and the hydrophobic moieties of the sites on a reversed phase packing. High to low salt gradients are used to effect this type of separation.

3.4.3 In some cases, such as with bonded stationary phases, the exact nature of the separation process is not fully established and it may be based on a combination of liquid-liquid and liquid-solid interactions.

3.4.4 In *Steric Exclusion Chromatography,* the stationary phase is a noninteractive porous solid, usually silica or an organic gel. In this case, separation is affected by the size of the sample molecules, where those which are small enough penetrate the porous matrix to varying extents and degrees while those that are largest are confined to the interstitial region of the particles. Thus, the larger molecules elute before the smaller molecules. See Practice D 3016 for further details of nomenclature for this technique.

3.5 In liquid chromatography, the composition of the mobile phase may be constant or changing during a chromatographic separation.

3.5.1 The term *Isocratic* may be used when the composition of the mobile phase at the column inlet is kept constant during a chromatographic separation.

3.5.2 The term *Gradient* is used to specify the technique when a deliberate change in the mobile phase operating condition is made during the chromatographic procedure. The change is usually in mobile phase composition, flow rate, pH, or temperature. The first-named change is called *Gradient Elution. Flow Programming* is a technique where the mobile phase linear velocity is changed during the chromatographic procedure. The changes are made to enhance separation or to speed elution of sample components, or both. Such changes in operating conditions may be continuous or step-wise.

3.6 In the standard modes of liquid chromatography, the stationary phase is more polar than the mobile phase. This is referred to as *Normal Phase Chromatography.* The opposite case is also possible, in which the mobile phase is more polar than the stationary phase. This version of the technique is called *Reversed-Phase Chromatography.*

3.7 *Planar Chromatography* comprises two versions: paper chromatography and thin-layer chromatography.

3.7.1 In *Paper Chromatography,* the process is carried out on a sheet or strip of paper. Separation is usually based on LLC in which water held on the cellulose fibers acts as the stationary phase. Separation based on LSC may also be utilized when the paper is impregnated or loaded with an interactive solid.

3.7.2 In *Thin-Layer Chromatography,* the solid stationary phase is utilized in the form of a relatively thin layer on an inactive plate or sheet.

3.7.3 In any version of planar chromatography, the mobile phase may be applied in a number of ways. In normal usage, *Ascending, Descending*, and *Horizontal Development*, the mobile phase movement depends upon capillary action. In *Horizontal Development*, the mobile phase may move predominantly linearly or radially. In *Radial Development*, the mobile phase is applied as a point source. Devices have been employed which accelerate the mobile phase movement on planar layers by pressure or centrifugal force.

3.7.4 The *Mobile Phase Front* is the leading edge of mobile phase as it traverses the planar media. In all forms of development, including radial, the local tangent to the *Mobile Phase Front* is everywhere normal to the local direction of development.

3.7.5 *Consecutive Developments* of planar media may be carried out after removal of the mobile phase from a previous development. If the consecutive development is accomplished in the same direction as previously, this is *Multiple Development.* If a second development is accomplished at a right angle to the first development, this is *Two-Dimensional Development.* Continuous development of planar media is possible by allowing evaporation of the mobile phase near the *Mobile Phase Front*.

3.7.6 *Impregnation* is the technique of applying a reagent to the planar media to effect an enhanced separation or detection. This impregnation is accomplished by dipping or spraying a reagent solution after the preparation of the medium, or by incorporating during the manufacturing process.

4. Apparatus

4.1 *Pumps*—The function of the pumps is to deliver the mobile phase at a controlled flow rate to the chromatographic column.

4.1.1 *Syringe Pumps* have a piston that advances at a controlled rate within a smooth cylinder to displace the mobile phase.

4.1.2 *Reciprocating Pumps* have a single or dual chamber from which mobile phase is displaced by reciprocating piston(s) or diaphragm(s). The chamber volume is relatively small compared to the volume of the column.

4.1.3 *Pneumatic Pumps* employ a gas to displace the mobile phase either directly or through a piston or collapsible container. The volume within these pumps may be large or

small as compared to the volume of the column.

4.2 *Sample Inlet Systems* represent the means for introducing samples into the column.

4.2.1 *Septum Injectors*—Sample contained in a syringe is introduced directly into the pressurized flowing mobile phase by piercing an elastomeric barrier. The syringe is exposed to pressure and defines the sample volume.

4.2.2 *Septumless Injectors*—Sample contained in a syringe is introduced into an ambient-pressure chamber, and the chamber is subsequently mechanically displaced into the pressurized flowing mobile phase. The syringe is not exposed to pressure and defines the sample volume.

4.2.3 *Valve Injectors*—Sample contained in a syringe (or contained in a sample vial) is injected into (or drawn into) an ambient-pressure chamber which is subsequently displaced into the pressurized flowing mobile phase. The displacement is by means of rotary or sliding motion. The chamber is a section (loop) of tubing or an internal chamber. The chamber can be completely filled, in which case the chamber volume defines the sample volume, or it can be partially filled, in which case the syringe calibration marks define the sample volume.

4.3 *Columns* consist of tubes that contain the stationary phase and through which the mobile phase flows.

4.3.1 *Separating Column* is the column on which the separation of the solutes is accomplished.

4.3.2 *Pre-column* is a column that has been used classically to precondition the mobile phase, placed between the pump and the injector. In the instance of its use with liquid-liquid separations involving coated stationary phases, such a column contained an excess of the coating phase to presaturate the mobile phase so it would not strip the same phase from the coated stationary phase during the separation. Its predominate use today is as a protector column for silica based column packing materials. It is filled with large particle silica which is slowly dissolved by polar, ionic mobile phases. By so doing, the silicate saturated mobile phase cannot dissolve the silica backbone of the analytical or preparative column.

4.3.3 *Guard Column* is a protector column placed between the injector and the separating column. The purpose of this column is to be the *final filter* for the sample, adsorbing unwanted sample components that otherwise might bind irreversibly to the separating column. It has a volume of no more than 1/20 the volume of the separating column. It may be filled with any material which will effectively remove the unwanted components without interfering with subsequent chromatographic processes.

4.3.4 *Concentrator Column* is a small column placed in-line at the loop injector for introducing a dilute sample which is collected into it before elution onto the separating column.

NOTE 4—Other terminology for this technique include, but are not limited to, trace enrichment column, collector column, and sample concentration column, but utilization of these terms is not recommended.

4.3.5 Column sizes with various internal diameters (ID) and lengths can be made. Larger columns present no problems concerning nomenclature, but columns with small internal diameters are now being used. As pointed out by Basey and Oliver[4] as many as nine terms (capillary, microcapillary, narrow bore capillary, micro, microbore, ultramicro, narrow bore, small bore, and small diameter) have been seen in the literature and with no clear distinction between them when the actual column ID is examined. It is recommended that all descriptive terms regarding column ID be discontinued, that is, *packed column, 1000 µm ID × 100 mm* or *open column, 250 µm ID × 1 m*.

4.3.6 *Column Inlet* is the end of a column where the mobile phase is introduced.

4.3.7 *Column Outlet* is the end of a column where the mobile phase exits.

4.3.8 *Frit* is the porous element placed at the ends of a chromatography column, or in a special device for in-line filtration to effect the removal of particulate material in the mobile phase or the sample solution.

4.4 *Detectors* are devices that respond to the presence of eluted solutes in the mobile phase emerging from the column. Ideally, the response should be proportional to the mass or concentration of solute in the mobile phase. Detectors may be divided either according to the type of measurement or the principle of detection.

4.4.1 *Bulk Property Detectors* measure the change in a physical property of the mobile phase passing from the column. Thus a change in the refractive index, conductivity, or dielectric constant of a mobile phase can indicate the presence of eluting components.

4.4.2 *Solute Property Detectors* measure the physical or chemical characteristics of the component eluting from the column. Thus, light absorption (ultraviolet, visible, infrared), fluorescence, and polarography are examples of detectors capable of responding in such a manner.

4.4.3 *Differential Detectors* measure the instantaneous proportion of eluted sample components in the mobile phase passing through the detector or their instantaneous rate of arrival at the detector.

4.4.4 *Integral Detectors* measure the accumulated quantity of sample component(s) reaching the detector.

4.4.5 The detectors used in liquid chromatography may also be based on a variety of other physical or chemical phenomena.

4.5 *Fraction Collectors* are devices for recovering time-separated fractional volumes of the column effluent. The fraction collectors may be operated manually or automatically. Automatic fraction collectors consist of a series of test tubes or flasks. Column effluent is carried to one of the vessels and after a measured volume is collected or a set period of time has passed, the system automatically places the next vessel into position to receive a corresponding aliquot.

4.6 *The Developing Chamber* is a closed or open container, for either conventional or continuous development, respectively. Customarily it is of relatively large internal volume, used to enclose the media used in paper or thin-layer chromatography and also the mobile phase. It may be lined with a porous paper (*Saturated Development*) or it may be unlined (*Unsaturated Development*). Paper or plate equilibration is also possible by standing the paper or thin layer plate in the

[4] *Journal of Chromatography*, No. 251, 1982, p. 265.

developing chamber containing the mobile phase for a given period of time before development without allowing the mobile phase to touch the paper or plate. If used for *Continuous Development*, the lid of the chamber is adjusted so the top portion of the thin layer plate can protrude past the lid allowing evaporation of the mobile phase near the solvent front. Automated instrumentation can effect this type of development by use of heated elements or air streams to force the evaporation of the mobile phase near the solvent front. A *Sandwich Chamber* has walls that are one half to one centimetre apart giving a relatively small internal volume. This type of developing chamber prohibits mobile phase vapors from getting onto the layer before the solvent front carries it throughout the layer effecting a different type of separation.

4.7 *Spotting Device* is a syringe or micropipet used to deliver a known volume of sample as a spot or streak to the paper or thin-layer media at the origin or near the beginning end of the planar media.

4.8 *Visualization Chamber* is a device in which the planar media may be viewed under ultraviolet light or sprayed with visualization reagents.

4.9 *Densitometer* is a device that allows portions of the developed paper or thin-layer media to be scanned with a beam of light of variable wavelength. The instrument in this manner is able to respond to differences in spot size and density in order to quantitate the separated compounds. The device may work in a transmission or reflectance mode.

5. Reagents

NOTE 5—In liquid chromatographic techniques the term "solvent" has been widely used to describe the mobile phase (that is, developing solvent, eluting solvent, solvent front). Due to the ambiguity of this term, its use is not recommended.

In various liquid chromatographic techniques the term "carrier" has been used to describe the solid on which the stationary phase is distributed or certain active groups involved in the separation process are bonded. Due to the similarity to the term "carrier gas" used as a synonym for the mobile phase in gas chromatography, the use of this expression is not recommended.

5.1 The *Mobile Phase* is the liquid used to sweep or elute the sample components along the planar surface or through the column. It may consist of a single component or a mixture of components. The term eluent is often used for the preferred *Mobile Phase*.

5.1.1 *Degassing* is the process of removing dissolved gases from the *Mobile Phase* before or during use. This can be accomplished by sparging (with helium), sonicating, heating, or applying a vacuum to the *Mobile Phase*.

5.2 The *Stationary Phase* is the active immobile material on the planar surface or within the column that retards the passage of sample components by one of a number of processes or their combination. There are three types of stationary phase: *Liquid Phases*, *Interactive Solids*, and *Bonded Phases.* Inert materials that merely provide physical support for the stationary phase are not part of the stationary phase.

5.2.1 The *Liquid Phase* is a stationary phase which has been sorbed (but not covalently bonded) to a solid support, paper sheet, or thin layer. Differences in the solubilities of the sample components in the liquid and mobile phase constitute the basis for their separation. Examples of materials that can be used as liquid phases are β,β′-oxydipropionitrile, silicone oil, and water.

5.2.2 The *Interactive Solid* is a stationary phase that comprises a relatively homogeneous surface on which the sample components sorb and desorb effecting a separation. Examples are silica, alumina, graphite, and ion exchangers.

5.2.3 The *Bonded Phase* is a stationary phase that comprises a chemical (or chemicals) that has been covalently attached to a solid support. The sample components sorb onto and off the bonded phase differentially to effect separation. Octadecylsilyl groups bonded to silica represent a typical example for a bonded phase.

5.2.3.1 A *Monomeric* phase is a bonded phase that has been attached to a support using a monofunctional silane reagent.

5.2.3.2 A *Polymeric* phase is a bonded phase that has been attached to a support using a di- or tri-functional silane reagent. The multifunctional reagent allows other cross-linking mechanisms to occur near the bonding region.

5.2.3.3 *Endcapping* is the process of bonding residual silanols not bonded by previous silanizing reactions through use of a smaller silanizing reagent such as trimethylchlorosilane.

5.2.3.4 *Coverage* is a relative measure of the amount of bonded phase on an inorganic support. It is usually described as $\mu mol/m^2$ or in terms of percent carbon.

5.3 The *Solid Support* is the inert material to which the stationary phase is sorbed (liquid phases) or covalently attached (bonded phases). It holds the stationary phase in contact with the mobile phase.

5.4 The *Column Packing* consists of all the material used to fill packed columns. There are two types: totally porous and pellicular.

5.4.1 A *Totally Porous Packing* is one in which the stationary phase is found throughout each porous particle.

5.4.2 A *Non-porous Packing* is one in which the stationary phase is found only on the porous outer shell of the otherwise impermeable particle. The previously used term, now obsolete, is pellicular packing.

5.5 *Solutes* are the sample components the separation of which is attempted on the column (column chromatography), paper sheet or thin-layer plate (planar chromatography) as they are swept or eluted by the mobile phase. These may be unretained (that is, not delayed) by the stationary phase in which case no separation is achieved, or they may be retained permanently. If partially retained, then separation to varying degrees may be accomplished.

5.6 *Binders* are the additives used to hold the stationary phase or solid support to the inactive plate or sheet in thin-layer chromatography. These may be calcium sulfate hemihydrate, starch, poly(vinyl alcohol), or others. Ideally, they play no part in the separation mechanism.

5.7 *Visualization* is that series of steps applied to planar media which may include evaporating off the mobile phase used for development, applying visualization reagents (one or a series, by spraying, vaporizing, or dipping), heating, and examination under visible or ultraviolet light to detect otherwise colorless solutes.

5.8 *Desalting* is the technique of removing low molecular

weight inorganic salts from higher molecular weight compounds, accomplished by steric exclusion chromatography or reversed phase chromatography or by dialysis.

6. Readout

6.1 The *Chromatogram* is the result of the separation of solutes through the process of chromatography.

6.1.1 If the separation is by means of column chromatography, the chromatogram is the graphic representation of the detector response versus retention time or retention volume as the solutes elute from the column and through the detector. An idealized chromatogram obtained with differential and integral detectors of an unretained and a retained component from a column is shown in Fig. 1.

6.1.2 If the separation is by means of planar chromatography, the chromatogram is the paper or thin-layer media itself on which the solute mixture has been placed and separated. An idealized chromatogram of a planar separation is shown in Fig. 2. The planar media may be passed under a densitometer in order to quantitate the separated compounds. The densitometer then produces a graphic representation of detector response versus distance traveled (retention time).

6.2 The definitions in 6.2.1 through 6.2.6 apply to chromatograms obtained directly by means of differential detectors or indirectly by differentiating the response of integral detectors.

6.2.1 A *Baseline* is the portion of a chromatogram recording the detector response when only the mobile phase emerges from the column.

6.2.2 A *Peak* is the portion of a chromatogram recording detector response when a single component, or two or more unresolved components, elute from the column.

6.2.3 The *Peak Base, CD* in Fig. 1, is the interpolation of the baseline between the extremities of a peak.

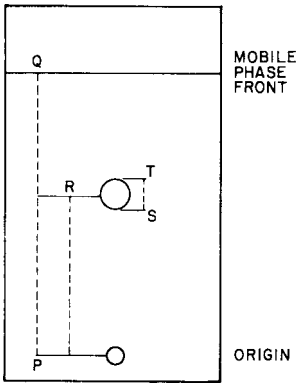

FIG. 2 Typical Planar Chromatogram

6.2.4 The *Peak Area, CHFEGJD* in Fig. 1, is the area enclosed between the peak and the peak base.

6.2.5 *Peak Height, EB* in Fig. 1, is the distance measured in the direction of detector response, from the peak base to peak maximum.

6.2.6 *Peak Widths* represent retention dimensions parallel to the baseline. *Peak Width at Base* or *Base Width, KL* in Fig. 1, is the retention dimension of the peak base intercepted by the tangents drawn to the inflection points on both sides of the peak. *Peak Width of Half Height, HJ* in Fig. 1, is the retention dimension drawn at 50 % of peak height parallel to the peak base. The *Peak Width at Inflection Points, FG* in Fig. 1, is the retention dimension drawn at the inflection points (= 60.7 % of peak height) parallel to the peak base.

6.2.7 *Asymmetry Factor* expresses the peak symmetry or non-Gaussian peak shape as a number. This number may indicate the chromatography occurring during the separation is other than ideal for any number of possible reasons (for

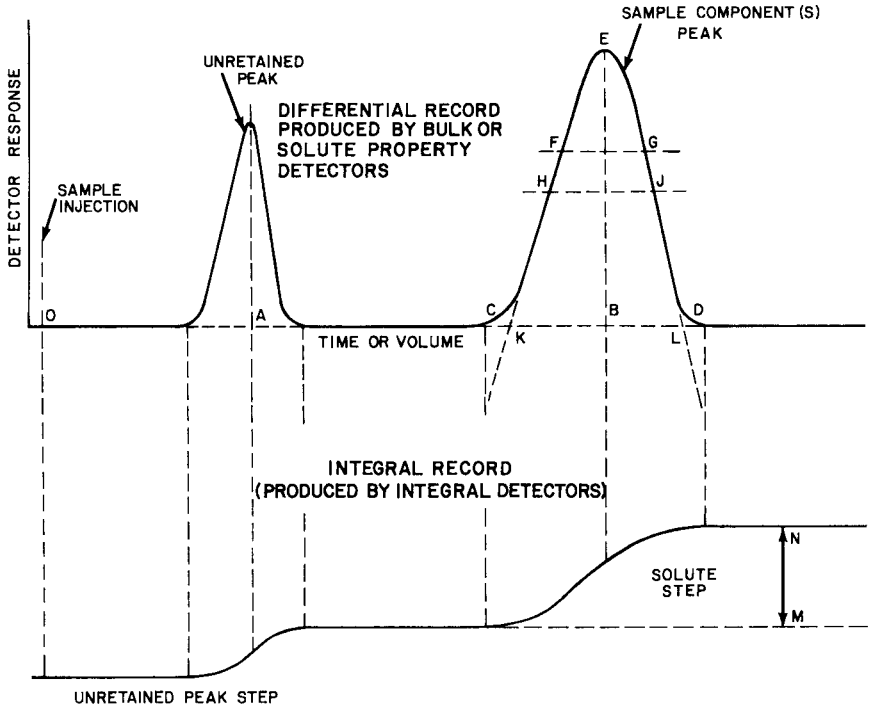

FIG. 1 Typical Chromatogram

example, thermodynamic, kinetic, inlet bed, or injection problems). It is defined as the ratio of the length of the trailing half of the peak divided by the length of the leading half of the peak, measured on a line perpendicular to a line dropped from the peak maximum, drawn 10 % of peak height above the baseline.

6.2.7.1 Another definition $A_s = (w_a + w_b)/2w_a$ is cited in USP XXI (p. 1230)[5] and USP Supplement 2 (p. 1905)[5] with the horizontal distances measured at 5 % of peak height, and is called tailing. The Federal Register, *50*, 9999 (1985)[6] used the same definition, measured at 10 % of peak height and called it asymmetry.

6.3 The definitions in 6.3.1 and 6.3.2 apply to chromatograms obtained with integral detectors, or by integration of the records obtained using differential detectors. In this mode of operation, as sample components pass through the detector, the baseline is displaced cumulatively.

6.3.1 A *Step* is the change in baseline position when a single component or two or more unresolved components elute.

6.3.2 The *Step Height, NM* in Fig. 1, is the distance, measured in the direction of detector response, between straight-line extensions of the baselines on both sides of a step.

6.4 The definitions in 6.4.1 through 6.4.3 apply to reading information from planar media.

6.4.1 The *Mobile Phase Distance*, *PQ* in Fig. 2, is the length of mobile phase traveling along the media from the center of the sample spot at the origin to the mobile phase front.

6.4.2 The *Solute Distance*, *PR* in Fig. 2, is the length of solute travel up the media from the center of the sample at the origin to the center of the solute spot. If the solute spot is other than circular, an imaginary circle is used whose diameter is the smallest diameter of the spot, and the center of this circle is taken as point *R*.

6.4.3 The *Spot Diameter, ST* in Fig. 2, which is equivalent to a peak width in chromatograms obtained by differential detectors, is the breadth of the solute spot after chromatography. As mentioned in 6.4.2, if the spot is not circular, the smallest diameter of the noncircular spot is used as the distance ST.

7. Retention Parameters, Symbols, and Units

7.1 Retention parameters, symbols, units, and their definitions or relationship to other parameters are listed in Table 1.

[5] Available from United States Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852.

[6] Available from Superintendent of Documents, U. S. Government Printing Office, Washington, DC 20402.

TABLE 1 Summary of Parameters, Symbols, Units and Useful Relationships in Liquid Chromatography

Parameter	Quantity Symbol	Unit	Definition or Relationship to Other Parameters[A]
Time	t	min	
Temperature of mobile phase	T	K	°C + 273.15 at the point where mobile phase flow is measured
Temperature of column	T_c	K	
Ambient temperature	T_a	K	
Column inlet pressure	P_i	Pa	
Column outlet pressure	P_o	Pa	
Pressure drop along the column	P	Pa	$P = P_i - P_o = Lu/B_o$
Relative column pressure	P		$P = P_i/P_o$
Ambient (atmospheric) pressure	P_a	Pa	
Column length	L	cm	
Column inside diameter	d_c	cm	
Average diameter of solid particles in the column	d_p	cm	
Pore radius	r_p	cm	
Interparticle porosity	ϵ		fraction of column cross section available for the moving phase
Column cross-sectional area	A_c	cm^2	$A_c = (d_c)^2\pi/4$
Volume of mobile phase in column + system	V_M	cm^3	$V_M = F_c t_M$
Interstitial volume of column	V_c	cm^3	In ideal case, assuming no extracolumn volume in system: $V_M = V_c$ In actual systems: $V_M = V_c + V_I + V_D$ where V_I is the volume between the effective injection point and the column inlet and V_D is the volume between the column outlet and the effective detection point
Molar volume	V_m	cm^3/mol	
Specific column permeability	B_o	cm^2	$B_o = \frac{d_p^2 \epsilon^3}{180(1-\epsilon)^2} = \frac{d_p^2}{1000}$
Flow rate of the mobile phase from the column	F_a	cm^3/min	measured at ambient temperature and pressure
Flow rate of mobile phase from the column, corrected to column temperature	F_c	cm^3/min	$F_c = Fa\frac{T_c}{T_a}$
Linear velocity of mobile phase	u	cm/s	$u = \frac{L}{60t_M} = \frac{F_a}{60\epsilon A_c}$
Optimum linear velocity of mobile phase	u_{opt}	cm/s	the value of u at the minimum of the HETP versus u plot; the value of u where the measured HETP is the smallest.

TABLE 1 *Continued*

Parameter	Quantity Symbol	Unit	Definition or Relationship to Other Parameters[A]
Viscosity of mobile phase	η	P [g/(cm·s)]	expressed at column temperature
Reduced mobile phase velocity	ν	...	$\nu = \frac{ud_p}{D_M}$
Diffusion coefficient of solute in mobile phase	D_M	cm^2/s	
Diffusion coefficient of solute in stationary phase	D_S	cm^2/s	
Retention time (total retention time)	t_R	min	time from sample injection to maximum concentration (peak height) of eluted compound
Mobile phase holdup time	t_M	min	observed elution time of an unretained substance
Adjusted retention time	t_R'	min	$t_R' = t_R - t_M$
Retention volume (total retention volume)	V_R	cm^3	$V_R = t_R F_c$
Adjusted retention volume	V_R'	cm^3	$V_R' = t_R' F_c$
Peak width at inflection points	w_i	cm	retention dimension between the inflection points (representing 60.7 % of peak height) of any single-solute peak
Peak width at half height	w_h	cm	retention dimension between the front and rear sides of any single-solute peak at 50 % of its maximum height
Peak width at base	w_b	cm	retention dimension between intersections of baseline with tangents to the points of inflection on the front and rear sides of any single-solute peak
Peak area	A	cm^2	
Distribution constant (partition coefficient)[B]	K		$K = \frac{\text{soluble concentration in the stationary phase}}{\text{solute concentration in the mobile phase}}$
Capacity ratio (partition ratio, capacity factor, mass distribution ratio)[B]	k		$k = t_R'/t_M = (t_R - t_M)/t_m = V_R'/V_M = (V_R - V_M)/V_M$
Number of theoretical plates[C]	n		$n = 16(t_R/w_b)^2 = 5.54(t_R/w_h)^2 = 4(t_R/w_i)^2$
Number of effective plates[C]	N		$N = 16(t_R'/w_b)^2 = 5.54(t_R'/w_h)^2 = 4(t_R'/w_i)^2 = n\left(\frac{k}{k+1}\right)^2$
Height equivalent to one theoretical plate[C]	h, HETP	cm	$h = L/n$
Height equivalent to one effective plate[C]	H, HEETP	cm	$H = L/N$
Reduced plate height[C]	h_r		$h_r = h/d_p$
Retention factor	R_f		a term used in paper and thin-layer chromatography $R_f = \frac{\text{distance moved by solute}}{\text{distance moved by mobile phase}}$ Sometimes the values are multiplied by 100.
R_M value	R_M		$R_M = \log[(1/R_f) - 1]$
R_s value	R_s		$R_s = R_f/R_{f(s)}$
Peak resolution (see Note 6, Note 6.2)	R_s		$R_s = \frac{2(t_{Rj} - t_{Ri})}{w_{bi} + w_{by}} \simeq \frac{t_{Rj} - t_{Ri}}{w_{hy}}$ where $t_{Rj} > t_{Ri}$
Relative retention	$r_{i,s}$		$r_{i,s} = t_{Ri}'/t_{Rs}' = K_i/K_s = k_i/k_s$
Relative retention (separation factor, separation ratio)	α		$\alpha = t_{R2}'/t_{R1}' = K_2/K_1 = k_2/k_1$ The symbol r is used to designate relative retention of a peak relative to the peak of a standard while the symbol Å is used to designate the relative retention of two consecutive peaks. By agreement, $t_{R2}' > t_{R1}'$ and thus, the value of α is always larger than unity while the value of r can be either larger or smaller than unity, depending on the relative position of the standard peak.
Number of theoretical plates required for a given resolution of peaks 1 and 2	n_{req}		$N_{req} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{k_2 + 1}{k_2}\right)^2$
Number of effective plates required for a given resolution of peaks 1 and 2	N_{req}		$N_{req} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2$
Weight-average molecular weight	M_W	g/mol	second moment of a polymer distribution
Number-average molecular weight	M_N	g/mol	first moment of a polymer distribution
Molecular weight distribution	MWD		weight (or number) fractions as a function of molecular weight
Integral molecular weight distribution	∫MWD		sum of weight fractions as a function of molecular weight
Differential molecular weight distribution	d(MWD)		relative abundance of a fraction as a function of molecular weight

TABLE 1 *Continued*

Parameter	Quantity Symbol	Unit	Definition or Relationship to Other Parameters[A]
Dispersity	d		a measure of the breadth of a molecular weight distribution
Hydrodynamic volume	V_h	cm^3/mol	a polymer molecular property proportional to M
Exclusion limit	V_h, max	cm^3/mol	maximum V_h that entered into pore
Solute designations (subscripts)	i		any solute
	j		a solute eluting after solute i
	s		a standard or reference solute
	1, 2		two consecutive solutes from which solute 2 elutes later than solute 1

[A] Peak position and width parameters refer to any one sample component unless otherwise shown by multiple-solute subscripts.

[B] In the literature, the symbol k is sometimes also used for the partition coefficient with the consequent use of k' (or K') for the capacity ratio. These usages are the result of individuals' preferences and have never been officially endorsed by the IUPAC or ASTM.

[C] The symbols used here for the various plate numbers and plate heights correspond to the long-standing nomenclature of ASTM in gas chromatography and also to the nomenclatures recommended by other standardizing groups. One can also find in the literature other meanings of the symbols and, therefore, it is important to always ascertain the meaning attributed in the particular publication. The most important differences from the usage recommended here are: (*a*) using *N* for the number of theoretical plates and *Neff* for the number of effective plates; (*b*) using *H* for the HETP, *Heff* for the HEETP, and *h* for the reduced plate height..

NOTE 6—From these the adjusted retention time, capacity ratio, number of theoretical plates, and relative retention are strictly speaking only meaningful in an isocratic, constant-flow system.

7.2 Fig. 1 can be used to illustrate some of the most common parameters measured from chromatograms obtained with differential detectors:

Elution time of unretained component	$= OA$
Retention time	$= OB$
Adjusted retention time	$= AB$
Capacity ratio	$= (AB)/(OA)$
Peak width at base	$= KL$
Peak width at half height	$= HJ$
Number of theoretical plates	$= 16[(OB)/(KL)]^2 = 5.54[(OB)/(HJ)]^2$
Relative retention (Note 7)	$= (AB)_j/(AB)_s$
Peak resolution (Note 7 and Note 8)	$= \frac{2(OB)_j - (OB_i)}{(KL)_i + (KL)_j} \simeq \frac{(OB)_j - (OB)_i}{(KL)_j}$

NOTE 7—Subscripts *i*, *j*, and *s* refer to some peak, a following peak, and a reference peak (standard), respectively.

NOTE 8—The second fraction may be used if peak resolution of two closely spaced peaks is expressed; in such as case $(KL)_i \simeq (KL)_j$.

7.3 Fig. 2 can be used to illustrate some of the most common parameters measured from chromatograms obtained for planar media:

Mobile phase distance	$= PQ$
Solute distance	$= PR$
Spot diameter	$= ST$
Retention factor (relative to mobile phase front) R_f	$= (PR)/(PQ)$
Retention factor (relative to a standard) (Note 6) R_s	$= (PR)_i/(PR)_s$
Number of theoretical plates	$= 16\,[(PR)/(ST)]^2$