



Standard Guide for Supercritical Fluid Chromatography Terms and Relationships¹

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1. Scope

1.1 This guide deals primarily with the terms and relationships used in supercritical fluid chromatography.

1.2 Since many of the basic terms and definitions also apply to gas chromatography and liquid chromatography, this guide is using, whenever possible, symbols identical to Practices E355 and E682.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

2. Referenced Documents

2.1 *ASTM Standards*:²

E355 Practice for Gas Chromatography Terms and Relationships

E682 Practice for Liquid Chromatography Terms and Relationships

3. Names of Techniques

3.1 *Supercritical Fluid Chromatography*, abbreviated as SFC, comprises all chromatographic methods in which both the mobile phase is supercritical under the conditions of analysis and where the solvating properties of the fluid have a measurable affect on the separation. Early work in the field was performed under a broader heading—*dense gas chromatography*. Related work in the field uses subcritical or near-critical conditions to affect separation.

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

3.3 In supercritical fluid chromatography, the pressure may be constant or changing during a chromatographic separation.

3.3.1 *Isobaric* is a term used when the mobile phase is kept at constant pressure. This may be for a specified time interval or for the entire chromatographic separation.

3.3.2 *Programmed Pressure Supercritical Fluid Chromatography* is the version of the technique in which the column pressure is changed with time during the passage of the sample components through the separation column. Isobaric intervals may be included in the pressure program.

3.4 In supercritical fluid chromatography, the temperature may be constant, or changing during a chromatographic separation.

3.4.1 *Isothermal Supercritical Fluid Chromatography* is the version of the technique in which the column temperature is held constant during the passage of the sample components through the separation column.

3.4.2 *Programmed Temperature Supercritical Fluid Chromatography* is the version of the technique in which the column temperature is changed with time during the passage of the sample components through the separation column. Isothermal intervals may be included in the temperature program.

3.5 In supercritical fluid chromatography, the density may be constant or changing during the chromatographic separation.

3.5.1 *Isoconfertic* is a term used when the density of the mobile phase is kept constant for a specified time or for the entire chromatographic separation.

3.5.2 *Programmed Density Supercritical Fluid Chromatography* is the version of the technique in which the column density is changed with time during the passage of the sample components through the separation column. Isoconfertic intervals may be included in the density program.

3.5.3 *Flow Programming* is a technique where the mobile phase linear velocity is changed during the chromatographic procedure. However, with fixed orifice restrictors, flow programming is more complex requiring an increase in pressure to effect an increase in linear velocity.

3.6 In supercritical fluid chromatography, the composition of the mobile phase may be constant or changing during a chromatographic separation.

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.6.1 The term *Isocratic* is used when the composition of the mobile phase is kept constant during a chromatographic separation.

3.6.2 The term *Gradient Elution* is used to specify the technique when a deliberate change in the mobile phase composition is made during the chromatographic procedure. *Isocratic* intervals may be included in the gradient program.

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).

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

4.2.1 *Direct Injection* is a sample introduction technique whereby the entire volume of sample is swept onto the head of the analytical column. Its use is most prevalent in packed column SFC.

4.2.2 *Split-Flow Injection* introduces only a portion of the sample volume onto the analytical column so as to prevent overloading of the column in open tubular SFC. This is achieved by the use of a splitter tee or similar contrivance, such that the incoming slug of sample is divided between the analytical column and a flow restrictor vented to waste. The amount of sample deposited on the column is a function of the ratio of the flow to the column versus the flow through this restrictor. This ratio can thus be adjusted for different samples and column capacities.

4.2.3 *Timed-Split (Moving-Split) Injection* achieves the same end result as split-flow injection. The volume of sample introduced onto the column is governed by the rapid back-and-forth motion of an internal-loop sample rotor in a valve. The time interval between the two motions determines the volume of sample injected, with shorter times delivering smaller volumes.

4.2.4 *On-Line Supercritical Extraction* is a means of directly introducing a sample or portion of a sample into a supercritical fluid chromatograph. The sample is placed in an extraction cell and extracted with the supercritical fluid. The extraction effluent containing the solutes of interest are ultimately transferred to the column by the action of switching or sampling valves. This can be accomplished with or without solute focusing (that is, using a suitable trap such as a cryogenic trapping).

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

4.3.1 *Packed Column Supercritical Fluid Chromatography* uses an active solid or a liquid that is chemically bonded to a solid and packed into a column, generally stainless steel or fused silica; as the stationary phase.

4.3.2 *Wall-Coated Open-Tubular Supercritical Fluid Chromatography* uses a liquid that is chemically bonded to the wall of an open-tubular column as stationary phase. Fused silica tubing columns, internal diameter (i.d.) > 100 μm , may shatter at pressures employed in SFC. A high degree of crosslinking is desirable to reduce stationary phase solubility in the mobile phase.

4.4 *Restrictors* are devices employed to maintain the pressure in the chromatographic system. The pressure of the supercritical fluid is usually reduced to ambient after passage through the restrictor. The mobile phase flow rate is determined by the restrictor dimensions or operation. The restrictor is placed before some types of detectors (for example, flame ionization, mass spectrometer) and after other types of detectors (for example, UV).

4.4.1 A *Linear Restrictor* is a length of small i.d. tubing of uniform bore. Linear restrictors are made of polyimidecoated fused silica tubing, or stainless steel or other tubing of the appropriate diameter. The amount of restriction provided is dependent upon both the length and i.d. of the tubing.

4.4.2 A *Tapered Restrictor* is a length of small i.d. tubing where one end has been reduced by drawing in a flame in the case of fused silica tubing, or crimped in the case of metal tubing.

4.4.3 An *Integral Restrictor (1)*³ consists of a length of fused silica tubing with one end closed by heating with a microtorch. This closed end is then ground until a hole with the desired initial linear velocity is obtained.

4.4.4 A *Converging-Diverging Restrictor (2)* has the wall of the tubing collapsed slightly near one end forming a constriction. This constriction is similar to a venturi in profile and the point of smallest diameter is located about 1 to 2 mm from the end of the tubing.

4.4.5 An *Orifice* is a type of restrictor which uses a metal disk or diaphragm with an appropriately sized opening. This type normally requires an adapter or holder specifically designed to couple the device to a detector.

4.4.6 A *Porous Frit Restrictor*⁴ consists of a length of fused silica tubing containing a porous plug at one end.

4.4.7 A *Back Pressure Regulator* consists of a diaphragm valve which can be adjusted to control the pressure maintained on its inlet (instrument) side. The outlet discharge pressure is nominally one atmosphere.

4.5 *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.

4.5.1 *Differential Concentration Detectors* measure the proportion of eluted sample component(s) in the mobile phase passing through the detector. The peak area is inversely proportional to the mobile phase flow rate.

³ The boldface numbers in parentheses refer to a list of references at the end of this standard.

⁴ Cortez, H., Pfeiffer, C., Richter, B., and Stevens, T. U. S., Patent No. 4 793 920, 1988.

4.5.2 *Differential Mass Detectors* measure the instantaneous mass of a component within the detector per unit time (g/s). The area under the curve is independent of the mobile phase flow rate.

5. Reagents

5.1 *Supercritical Fluid* is a fluid state of a substance intermediate between a gas and a liquid. A supercritical fluid may be defined from the accompanying phase diagram (Fig. 1). The supercritical fluid region is defined by temperatures and pressures, both above the critical values. A subcritical fluid (or liquid) is a compound that would usually be a gas at ambient temperature but is held as a liquid by the application of pressure below its supercritical point.

5.1.1 The *Critical Temperature* is the temperature above which a substance cannot be liquefied or condensed no matter how great the applied pressure.

5.1.2 The *Critical Pressure* is the pressure that would just suffice to liquefy the fluid at its critical temperature.

5.1.3 The *Reduced Pressure* is the ratio of the working pressure to the critical pressure of the substance.

5.1.4 The *Reduced Temperature* is the ratio of the working temperature to the critical temperature of the substance.

5.1.5 The *Density* of a supercritical fluid (the weight per unit volume of the fluid) in chromatographic separations is calculated from an empirical equation of state.

5.2 A *Modifier or co-solvent* is a substance added to a supercritical fluid to enhance its solvent strength, usually by increasing the polarity of the mobile phase, or binding to active sites on a stationary phase.

5.3 The *Stationary Phase* is composed of the active immobile materials within the column that selectively retard the passage of sample components. Inert materials that merely provide physical support or occupy space within the columns are not part of the stationary phase.

NOTE 1—Extremely porous stationary phases may exhibit exclusion phenomenon in addition to adsorptive interactions.

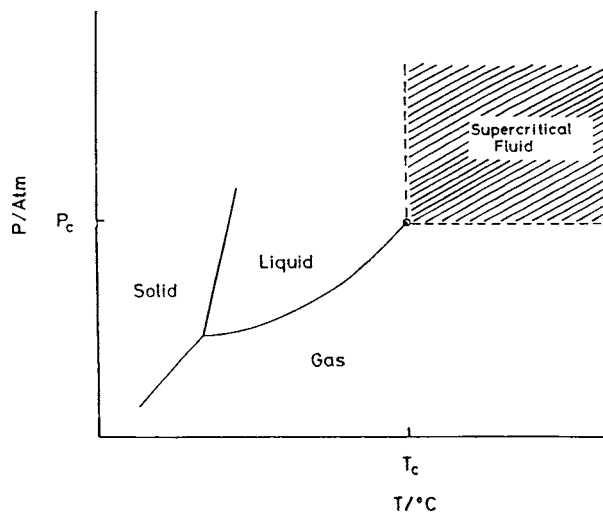


FIG. 1 Phase Diagram

5.3.1 An *Interactive Solid* is a stationary phase material with bulk homogeneity where the surface effects separation by adsorptive interactions. Examples are silica and alumina.

5.3.2 A *Bonded Phase* is a stationary phase that has been covalently attached to a solid support. The sample components partition between the stationary and mobile phases which results in separation. Octadecylsilyl groups bonded to silica gel particles and polydimethylsiloxane (or dimethyl polysiloxane) bonded to deactivated fused silica column wall represent examples for packed column and open tubular column phases, respectively.

5.4 The *Solid Support* is the inert material that holds the stationary phase in intimate contact with the mobile phase. It may consist of porous or impenetrable particles or granules or the interior wall of the column itself, or a combination of these.

5.5 The *Column Packing* consists of all the material used to fill packed columns, including the solid support and the bonded phase or the interactive solid.

5.6 *Solutes* are the sample components that are introduced into the chromatographic system and are transported by the mobile phase and elute through the column. Some solutes may be unretained.

6. Readout

6.1 A *Chromatogram* is a plot of detector response against time or effluent volume. Idealized chromatograms obtained with a differential detector for an unretained substance and one other component are shown in Fig. 2.

6.2 The definitions in 6.2.1-6.2.6 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 that portion of a chromatogram where no detectable sample components emerge from the column.

6.2.2 A *Peak* is that portion of a chromatogram where a single detectable component, or two or more unresolved detectable components, elute from the column.

6.2.3 The *Peak Base*, CD in Fig. 2, is the interpolation of the baseline between the extremities of a peak.

6.2.4 The *Peak Area*, CHFEGJD in Fig. 2, is the area enclosed between the peak and the peak base.

6.2.5 *Peak Height*, EB in Fig. 2, is the perpendicular distance measured in the direction of detector response, from the peak base to peak maximum.

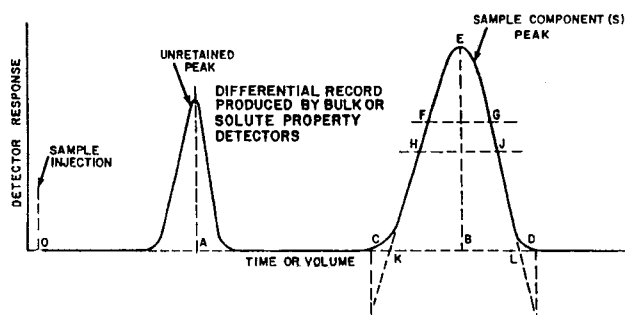


FIG. 2 Typical Chromatogram

6.2.6 *Peak Widths* represent retention dimensions parallel to the baseline. Peak width at base or base width, KL in Fig. 2, 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 at half height, HJ in Fig. 2, is the retention dimension drawn at 50 % of peak height parallel to the peak base. The peak width at inflection point, FG in Fig. 2, is the

retention dimension drawn at the inflection points (+60.7 % of peak height) parallel to the peak base.

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 (3, 4).

TABLE 1 Summary of Parameters, Symbols, Units and Useful Relationships in Supercritical Fluid Chromatography 1–2

Parameters	Quality Symbol	Unit	Definition or Relationship to Other Parameters ^A
Time	t	min	
Temperature of mobile phase	T	K	$^{\circ}\text{C} + 273.15$ at the point where mobile phase flow is measured
Absolute temperature of supercritical fluid	T	K	$^{\circ}\text{C} + 273.15$
Critical temperature of supercritical fluid	T_c	K	
Reduced temperature of supercritical fluid	T_r		$T_r = T/T_c$
Pressure of supercritical fluid	P	Pa	
Critical pressure of supercritical fluid	P_c	Pa	
Reduced pressure of supercritical fluid	P_r		$P_r = P/P_c$
Density of supercritical fluid	ρ	g/cm^3	
Specific permeability of column	B_o	cm^2	For open tubular columns: $B_o = \frac{d_c^2}{32}$ For packed columns: $B_o \approx \frac{d_p^2}{1000}$ For packed capillary columns: $B_o \approx \frac{d_p^2}{300}$
Phase ratio of column	β		For open tubular columns: $\beta = r/2d_f$
Ambient temperature	T_a	K	
Column inlet pressure	P_i	Pa	
Column outlet pressure	P_o	Pa	
Pressure drop along the column	ΔP	Pa	$\Delta P = P_i - P_o = Lu/B_o$
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	
Stationary phase thickness	d_f	cm	
Column radius	r	cm	
Pore radius	r_p	cm	
Column cross-sectional area	A_c	cm^2	$A_c = (d_c)^2\pi/4$
Molar volume	V_m	cm^3/mol	
Average linear velocity of mobile phase	\bar{u}	cm/s	$\bar{u} = \frac{L}{60t_M}$ (linear velocity is usually measured at the initial chromatographic conditions)
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
Viscosity of mobile phase	η	$\text{P}(\text{g}/\text{cm}\cdot\text{s})$	Pa·s expressed at fluid temperature or reduced temperature
Reduced mobile phase velocity	v		$v = \frac{\bar{u}dp}{D_M}$ for packed columns $v = \frac{\bar{u}d_c}{D_M}$ for open tubular columns
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	$t_R' = t_R - t_M$
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		$K = \frac{\text{solute concentration in the stationary phase}}{\text{solute concentration in the mobile phase}}$
Capacity ratio (partition ratio, capacity factor, mass distribution ratio) ^B	k		$k = t_R'/t_M = (t_R - t_M)/t_M$
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		$N = 16(t_R'/w_b)^2 = 5.54(t_R'/w_h)^2 = 4(t_R'/w_i)^2 = n \left(\frac{k}{k+1} \right)^2$

TABLE 1 Continued

Parameters	Quality Symbol	Unit	Definition or Relationship to Other Parameters ^A
Height equivalent to one theoretical plate ^C	<i>h</i> , HETP	cm	$h = L/n$
Height equivalent to one effective plate ^C	<i>H</i> , HEETP	cm	$H = L/N$
Reduced plate height ^C	<i>h_r</i>		$h_r = h/d_p$ for packed columns = h/d_c for open tubular columns
Peak resolution (see Note 3)	<i>R_s</i>		$R_s = \frac{2(t_{Rj} - t_{Ri})}{W_{bi} + W_{bj}} = \frac{t_{Rj} - t_{Ri}}{W_{bj}}$ where $t_{Rj} > t_{Ri}$
Relative retention	<i>r_{i,s}</i>		$(r_{i,s} = t_{Ri}/t_{Rs} = K_i/K_s = k_i/k_s)$
Relative retention (separation factor, separation ratio)	α		$\alpha = t_{R2}/t_{R1} = K_2/K_1 = k_2/k_1$ The symbol <i>r</i> is used to designate relative retention of a peak relative to the peak of a standard while the symbol <i>A</i> 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 <i>Y</i> 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	<i>n_{req}</i>		$n_{req} = 16R_s^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{k_2 + 1}{k_2} \right)^2$
Number of effective plates required for a given resolution of peaks 1 and 2	<i>N_{req}</i>		$N_{req} = 16R_s^2 \left(\frac{\alpha}{\alpha - 1} \right)^2$

^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 Committee E-19.
^C The symbols used here for the various plate numbers and plate heights correspond to the long-standing nomenclature of ASTM Committee E-19 on 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 *N_{eff}* for the number of effective plates; (b) using *H* for the HETP, *H_{eff}* for the HEETP, and *h* for the reduced plate height.

NOTE 2—From these the adjusted retention time, capacity ratio, number of theoretical plates, and relative retention are, strictly speaking, only meaningful in isocratic, isobaric, isoconfertic, isothermal, and constant-flow systems.

7.2 Fig. 2 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 3) = $(AB)/(AB)_s$

Peak Resolution (Note 3, Note 4) =

$$\frac{2[(OB)_j - (OB)_i]}{(KL)_i(KL)_j} \approx \frac{(OB)_j - (OB)_i}{(KL)_j}$$

NOTE 3—Subscripts *i*, *j*, and *s* refer to some peak, a following peak, and a reference peak (standard), respectively.

NOTE 4—The second fraction may be used if peak resolution of two closely spaced peaks is expressed; in such a case $(KL)_i = (KL)_j$.

8. Equations of State

8.1 Dense gases deviate considerably from ideal behavior and several equations of state have been used to express the relationship between the state functions. One such equation uses the compressibility factor to account for the deviation. The compressibility factor is given in the following expression.

$$Z = \frac{PV}{RT} \tag{1}$$

8.1.1 In this equation, *R* is the gas constant, *P* is pressure, *T* is temperature in *K*, and *V* is the molar volume of gas. Three parameter correlations use functions of reduced variables *T_r*, *P_r*, and the Pitzer (5) acentric factor, ω .

$$Z = Z^{(0)}(T_r, P_r) + \omega Z^{(1)}(T_r, P_r) \tag{2}$$

8.1.2 Values of ω , $Z^{(0)}$, and $Z^{(1)}$ have been tabulated (6). Substituting this relationship into the former equation and using the definitions for *P* and *T* in terms of reduced variables an equation relating density to pressure and temperature is finally obtained.

$$\rho = \frac{P_r P_c M}{R(Z^{(0)} + \omega Z^{(1)})T_r T_c} \tag{3}$$

In the preceding equation *M* is the molecular weight of the gas.

8.2 In addition to three parameter correlations, cubic equations of state have also been used. The general form of these are shown below.

$$P = \frac{RT}{V - b} - \frac{a}{V^2 + ubV + wb^2} \tag{4}$$

The parameters *u*, *w*, *b*, and *a* for three common cubic equations are tabulated in Table 2.

TABLE 2 Constants for Cubic Equations

 NOTE 1—For SRK equation $f\omega = 0.48 + 1.574\omega - 0.176\omega^2$

 NOTE 2—For PR equation $f\omega = 0.37464 + 1.54226\omega - 0.26992\omega^2$

Equation	u	w	b	a
Van der Waals	0	0	$\frac{RT_c}{8P_c}$	$\frac{27R^2T_c^2}{64P_c}$
SRK (7)	1	0	$\frac{0.08664RT_c}{P_c}$	$\frac{0.42748R^2T_c^2}{P_c} [1 + f\omega (1 - T_r^{1/2})]^2$
PR (8)	2	-1	$\frac{0.07780RT_c}{P_c}$	$\frac{0.45724R^2T_c^2}{P_c} [1 + f\omega (1 - T_r^{1/2})]^2$

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