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RETENTION PARAMETERS IN CHROMATOGRAPHY

(IUPAC Recommendations 2001)

PART A. HOLD-UP VOLUME CONCEPT IN COLUMN CHROMATOGRAPHY

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PART B. RETENTION PARAMETERS IN GAS CHROMATOGRAPHY

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Abstract: The paper presents a revision of terms in the IUPAC "Nomenclature for Chromatography", *Pure and Applied Chemistry,* **65**, 819–872, 1993. The terms revised pertain to hold-up volumes in gas, liquid, and supercritical-fluid chromatography, as well as to basic retention parameters, especially in gas chromatography. A number of related and derived definitions are described, including definitions of the terms "chromatographic process" and "chromatographic phase system". A number of the original terms were found to be misleading or superfluous, including such terms as corrected retention time, net retention time, total retention volume (time), and specific retention volume at 0 °C, and their use is strongly discouraged

In Part A, the concept of the hold-up volume in chromatography is discussed. The paper also compares methods described in the literature to determine the hold-up volume.

In Part B, retention parameters in gas chromatography are discussed with the aim of (i) emphasizing the physical meaning of the terms and (ii) specifying the temperatures and pressures for the terms for gas volumes and flow rates.

The appendix presents revised recommendations for the terminology of some items, as well as those that are not recommended.

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Retention parameters in chromatography

(IUPAC Recommendations 2001)

Part A. Hold-up volume concept in column chromatography

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1. INTRODUCTION

The IUPAC document "Nomenclature for Chromatography (IUPAC Recommendations 1993)" (NC) [1] presents a description of the hold-up volume (described in NC 3.7.03) that is now considered to require a more precise definition. This paper looks at a number of related and derived definitions for retention parameters and proposes revised recommendations for their terminology, including definitions of the

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terms "chromatographic process" and "chromatographic phase system". This last term is now introduced to describe what should be termed "chromatographic system" (the system where a chromatographic process occurs). The term chromatographic system has been used in the original IUPAC publication [1], both as referring to the phase system (see *Stationary Phase*, NC 1.1.05) and as a reference to the instrumental setup necessary to carry out chromatographic separations (NC 2). The latter use has gained widespread acceptance among chromatographers. Therefore, in order to avoid unnecessary confusion, the new term is now proposed. The present paper also compares methods described in the literature to determine the hold-up volume.

2. DESCRIPTION OF TERMS

2.1 Chromatographic phase system and chromatographic process

A *chromatographic phase system* is formed by at least two immiscible phases in contact with each other. Occasionally, more than two phases are present, but this will not introduce any changes in the descriptions given here, so only two phases will be considered. One of them (the *mobile phase* NC 1.1.06) is continuously moving in a constant direction relative to the other (the *stationary phase*), which is that part of the system responsible for the retention of the analytes [2]. *Chromatography* (NC 1.1.01) is the name given to the method of separation carried out in a chromatographic phase system. A *chromatographic process* is the dynamic distribution between phases that takes place when an analyte (or a mixture of analytes) is carried along the stationary phase by the movement of the mobile phase. The components of the chromatographic phase system are often, but not necessarily, contained in a tube, and the whole (the chromatographic phase system and the tube) is called the column. The separation technique carried out in such a column is termed *column chromatography* (NC 1.3.01), and will be the technique to which this paper will refer.

A chromatographic process is initiated when a sample (a substance or a mixture of substances), generally mixed with the mobile phase, comes in contact with the chromatographic phase system and starts to move, carried by the mobile phase. This is the real injection of the sample in the chromatographic phase system. However, the term "sample injection" is currently applied to the introduction of the sample into the mobile phase through the injection device of the *chromatograph* (NC 1.1.04), often located upstream of the chromatographic phase system. "*Initiation time*" should be used instead, to refer to the start of the chromatographic process. Throughout this paper, the sample will be considered to have only one component (hereafter, the analyte), and the technique referred to will be column chromatography. The analyte distributes itself between the two phases, and the movement of the mobile phase carries the analyte along the column. The chromatographic process ceases at the point when the analyte leaves the column. This description corresponds to an example of what is termed "*elution chromatography*" (NC 1.2.03). Due to the various dynamic processes that take place inside the column, the analyte is gradually diluted with the mobile phase as it is being transported along the column, forming a band. In the discussion that follows it is assumed that a linear distribution isotherm exists between the two phases [3]. While travelling along the column, the analyte is assumed to present a Gaussian distribution of concentration, the maximum of this distribution representing the position of the analyte band. Accordingly, for the sake of this discussion, "an analyte leaves the column" when the point of maximum concentration of the distribution zone reaches the exit of the column.

2.2 Retention volume, adjusted retention volume, and hold-up volume

The volume of mobile phase that leaves the column, from the moment of the entrance of the analyte into the chromatographic phase system (the initiation time) to the moment in which the analyte leaves, is called the "*retention volume*" of the analyte. This description does not correspond to the definition of "*total retention volume*" given in the IUPAC document [1] (NC 3.7.05). The difference will be shown

in section 2.3. If the analyte is not retained by the stationary phase, then the volume of mobile phase that leaves the column from the initiation time to the moment at which the analyte leaves the chromatographic phase system (that is, the retention volume of an unretained substance) is called the "*hold-up volume*" of the chromatographic phase system. The description in NC 3.7.03 [1] is different from what is given here. Sometimes, this volume is referred to with the term "*dead volume*" (NC 3.2.13.1); this term, however, is ambiguous, and its use is discouraged [1].

During the chromatographic process, there will be molecules of the retained analyte in both the mobile and the stationary phases. Any one of those molecules will spend part of its time in the stationary phase (dissolved, adsorbed, etc.) not moving along the column (diffusion in the stationary phase will be ignored); and the rest of the time in the mobile phase, moving along the column at the same linear velocity as the mobile phase. The volume of mobile phase used to transport a given molecule of the retained analyte while it is in the mobile phase, is the same as the volume used to transport the molecules of the mobile phase itself along the column: the hold-up volume. The retention volume of an analyte is, thus, composed of two parts: the volume of the mobile phase used to transport it along the column while in the mobile phase, and the volume of the mobile phase that left the column during the time the molecules of the analyte were stationary in the stationary phase. The latter volume is called the "*adjusted retention volume*" (NC 3.7.07) of the analyte, and is the chromatographic parameter that is related to the thermodynamics of the processes that takes place. Retention volumes are the parameters that describe the chromatographic process. The symbols accepted to refer to the three volumes just mentioned are V_R for the retention volume, V_M for the hold-up volume, and V_R' for the *adjusted retention volume* [1]. In *liquid chromatography* (LC), the term "void volume" (V_0) is normally applied to the hold-up volume. However, the symbol V_0 has also been applied to the "*interparticle volume*" or "*interstitial volume*" of the column (NC 3.2.11), which does not correspond to the hold-up volume of the chromatographic phase system (see later), so the usual symbol V_M is the recommended symbol to be used for the hold-up volume and the use of V_0 in this context is discouraged.

2.3 Extra-column volume

The mixing of the analyte with the mobile phase normally occurs before they reach the column (i.e., in the injection device of the chromatograph). Accordingly, a certain amount of mobile phase leaves the column in the time between sample injection and the moment the analyte enters the chromatographic phase system (the initiation time). In addition, when the mobile phase leaves the column, it is usually carried to a detector using some sort of connection (capillary tubes and end pieces), and a further volume of mobile phase is required before the eluted sample is detected. As a result, the volume of mobile phase that leaves the column between sample injection and detection is the sum of three terms: the adjusted retention volume (V_R'), the hold-up volume (V_M), and the volumes of the injector, detector, and connections, known collectively as the "*extra-column volume*" V_{ext} (NC 3.2.13). Extra-column volumes do not have any chromatographic meaning and should be avoided or minimized wherever possible. In the past, the extra-column volume has been considered as part of both the retention volume and the hold-up volume [1]. From the description above, it is clear that the extra-column volume is not part of the chromatographic phase system and should not be included as part of those volumes. Therefore, the experimentally measured retention volume from injection to detection, (the "*gross retention volume*", V_R^g [4]), will be:

$$V_R^g = V_R' + V_M + V_{ext} = V_R + V_{ext} \tag{1}$$

The retention volume of an unretained substance, including the extra-column volume, would be the "*gross hold-up volume*", V_M^g.

The existence of extra-column volumes has often been ignored by practicing chromatographers when calculating system characteristics, such as the phase ratio (NC 3.2.17), the retention factor (NC 3.7.12) or the average linear mobile-phase velocity (NC 3.6.05). According to the description

above, this is an incorrect practice. The errors involved will depend on the relative value of the various volumes of eq. 1. In *gas chromatography* (GC) (NC 1.2.02), modern apparatuses for capillary column gas chromatography (CCGC) achieve a good experimental approximation to a separation in which there is no extra-column volume if a large split ratio in a low volume injector is used, the column end directly enters the detector and a reasonable amount of auxiliary or make-up gas is employed to minimize the effect of the detector volume. In LC, V_{ext} can be found by an independent experiment (without the column).

2.4 Mobile-phase volume in the chromatographic phase system

The hold-up volume is defined here as the volume of mobile phase that leaves the column during the transport of an unretained substance along the column. Normally, this volume is equal to the total volume of mobile phase in the chromatographic phase system. It includes both the interparticle (exclusion) volume in packed columns and the mobile phase inside the pores of the packing material. In some chromatographic techniques, especially in *gas–solid chromatography* (GSC) (NC 1.4.01), LC, and *supercritical-fluid chromatography* (SFC) (NC 1.4.04), but perhaps also in packed-column *gas–liquid chromatography*, GLC (NC 1.4.01), a certain amount of mobile-phase material inside the column could be inaccessible to the analyte, while remaining accessible to other molecules of the mobile phase. The inaccessibility to molecules of the analyte is referred to as an exclusion phenomenon, attributed to sterical or electrostatic reasons. This phenomenon is the basis of *size exclusion chromatography* (SEC) and *ion exclusion chromatography* (IEC) (NC 1.5.04). These special cases are not considered here. It must be specially mentioned here that a portion of the mobile phase inside the column may be immobilized (it does not move), but may be accessed by the molecules of the analyte. Calculations based on the diffusion rates of solute molecules in liquids (diffusion rates in gases are orders of magnitude larger) indicate that under chromatographic conditions the analyte molecules will reach the bottom of any normal pore in the time taken by the analyte band to pass over it. Similarly, mobile-phase pools formed on the solid surface of particles may be accessible to analytes. These portions of immobilized mobile phase will slow down the overall movement of any analyte, retained or not. They take part in the chromatographic process. If an adequate probe is available (a truly unretained analyte), the measure of the volume of mobile phase that leaves the column during its elution will include the effects of the interactions within these immobilized portions.

The volumes of mobile phase mentioned so far have been described as "volumes measured at the column outlet". The reporting of experimental values of V_M is not always simple. Liquids are incompressible, so their volume does not depend on pressure and very little on temperature. However, the volume of a given amount of gas, or fluid under supercritical or near supercritical conditions, will depend on both temperature and pressure, and these must be recorded when volumes are reported and used. In such cases, in order to specify chromatographic conditions, V_M must be expressed at the column temperature and ambient pressure (NC 3.6.04.2 and NC 3.7.03). The correction factors that must be applied in GC, to express mobile-phase volume under different conditions have been discussed intensively [1,5–14], and the recommended names and definitions are summarized by V. A. Davankov in Part B [14].

Mobile-phase volumes have been described in the past as "volume that entered the column...", "volume that passed any cross-section of the column...", "volume that left the column...", "volume used to transport...". All of these expressions are equivalent, as long as the effects of pressure and temperature are not forgotten. Other expressions such as "volume inside the column..." or "volume that moves inside the column...", although normally correct, do not always describe the hold-up or retention volumes and should be avoided.

2.5 Retention parameters based on time

A chromatogram is often the representation of the variation, with time or volume, of the amount of the analyte in the mobile phase exiting the chromatographic column (measured at the detector device). Quite often, the chromatographic process is carried out isothermally under conditions of constant flow rate of the mobile phase. Under these circumstances, retention volumes may be related to retention times. Therefore, "*retention time*" (t_R), "*adjusted retention time*" (t_R'), "*hold-up time*" (t_M) and "*extra-column time*" (t_{ext}), may be used instead of the corresponding volumes. Times may be measured with reasonable precision with electronic integrators or data systems, as long as the sampling rate of the computer/integrator is adequate. However, when measuring times, an error might be introduced at the moment of initiating the time count. This time error will have a corresponding volume error. For simplicity, this error will not be considered here. The experimental retention time of an analyte, including all possible errors, will be denoted as t_R^g ("*gross retention time*"). The time corresponding to the retention of an unretained substance would be t_M^g ("*gross hold-up time*"), sometimes referred to as t_0. The latter symbol, however, is normally reserved to denote the retention time of an unretained compound in exclusion chromatography (EC, NC 1.5.04). Its use in connection with elution chromatography is discouraged.

The thermodynamically appropriate retention parameter in chromatography is the retention volume; but as times and not volumes are measured directly, times are normally used in gas chromatography. In liquid chromatography, conversion from time to volume is straightforward, and the normal practice is to use and mention volumes. It must be remembered that for a given chromatographic phase system at a defined temperature, retention volumes and the hold-up volume are independent of flow rate, but the corresponding times are not.

In any discussion of the hold-up time, two concepts must be clearly distinguished: the hold-up time itself, t_M, as defined earlier, and the point of the chromatogram corresponding to the time when a nonretained compound would appear, t_M^g, gross hold-up time. According to the previous description, we have:

$$t_M^g = t_M + t_{ext} \tag{2}$$

The difference between t_M and t_M^g has frequently been ignored in the past. If t_{ext} is carefully minimized, the values become essentially equivalent.

3. THE UTILITY OF THE HOLD-UP TIME (VOLUME)

Hold-up times or volumes are used in chromatography for various purposes. In those expressions in which t_M is used as a term, the exact point of the chromatogram where the unretained analyte should appear (t_M^g) must be known exactly. An example would be the expression used to calculate adjusted retention times: $t_R' = t_R^g - t_M^g$. In cases where the hold-up time is used as a factor of the expression, the correct value of t_M must be used. An example will be the retention factor, k, which may be obtained as follows:

$$k = (V_R - V_M)/V_M = V_R' / V_M = t_R'/t_M \tag{3}$$

In this case the retention factor may not be obtained with the expression: $k = (t_R^g - t_M^g) / t_M^g$ except in the particular case in which $t_M = t_M^g$. However, the expression: $k = (t_R^g - t_M^g) / t_M$ is correct.

Some chromatographic and thermodynamic parameters may be calculated accurately using the gross hold-up time: adjusted, corrected, net and specific retention volumes (V_R', V_R°, V_N, V_g); adjusted retention times (t_R'); distribution constant or partition coefficient (K); theoretical and effective plate number of the column (N, N_{eff}); separation factor (α); relative retention of peaks (r); resolution of two peaks (R_s); peak capacity (n), separation number (SN) or Trennzahl (TZ); Kovàts' retention indices (I). Other system-dependent parameters may not be calculated with accuracy, unless the correct value of t_M

or V_M is known: retention factor (k); retardation factor (R); phase ratio (β); hold-up volume (V_M) if deduced from t_M and the flow rate; average linear mobile-phase velocity ($\bar{u}$). Definitions and expressions used to calculate all these parameters may be found in refs. 1,14.

4. METHODS USED TO ESTIMATE THE HOLD-UP TIME (VOLUME)

The methods used for hold-up time or volume determination depend on the physical state of the mobile phase. Accordingly, those for gas and liquid mobile phases will be treated separately. Direct methods (the experiment produces the value of V_M or t_M directly) and indirect methods (results of the experiment are used to calculate the value of V_M or t_M) have been proposed to find the hold-up time (volume). Not all methods produce the same value for V_M or t_M for a given chromatographic phase system.

4.1 Gas chromatography

In gas chromatography, all methods proposed to estimate hold-up time or volume use experimental retention times and the methods are intended for hold-up time estimation. However, none of the methods gives a value of the hold-up time; what they really give is the point on the chromatogram where an ideal non-retained substance would appear (t_M^g). All methods assume that sufficient experimental precautions have been taken to assure the absence of time errors due to the existence of extra-column volumes or to other sources of error: none of them corrects these possible errors. In the following paragraphs, the words "hold-up time" will be used, but it is always "gross hold-up time" (t_M^g) that is measured. It has been mentioned that the latter value is adequate for many applications.

4.1.1 Direct method

The best estimate of the hold-up time should be obtained by the injection of a substance that is not retained by the stationary phase, and that may be monitored by the detector. The retention time of such a substance would show the exact point of the chromatogram to be used for adjusting retention times but not the true value of t_M, unless the systematic errors mentioned above have been avoided. In GC, such an ideal substance does not exist [15,16], although some permanent gases may be used without much error, if an appropriate detector is available. Methane is not recommended as a hold-up time indicator. It may be used, however, to calculate chromatographic or thermodynamic parameters of analytes with large retention factors.

4.1.2 Indirect methods

The absence of a true nonretained substance for direct estimation of the hold-up time, and the widespread use of the flame ionization detector has forced the development of indirect ways of estimation. Reviews may be found in a paper by Smith *et al.* [17] or in the book by Pacáková and Feltl [18]. Only a few of the procedures currently used will be mentioned in this paper. Sometimes, the hold-up time calculated with these methods has been referred to as "*mathematical hold-up time*", denoted with the symbol t_{MM}.

4.1.2.1 Linearity methods

The methods that have found widest acceptance are those based on the linearity of representation of the logarithm of the adjusted retention times for the members of a homologous series (preferably *n*-alkanes) vs. carbon number, for chain lengths of a minimum of five or six carbon atoms ("the semilog plot"). It is generally accepted that the linearity does not apply to the whole homologous series, but only to a range of chain lengths that seems to depend on the experimental conditions [19]. Early methods relied on the experimental retention times of three homologues. For evenly spaced homologues:

$$t_{MM} = t_{R,2} - \frac{(t_{R,2} - t_{R,1})(t_{R,3} - t_{R,2})}{(t_{R,3} - t_{R,2}) - (t_{R,2} - t_{R,1})} \tag{4}$$

where $t_{R,X}$ (x = 1, 2, and 3) is the experimental retention time of the homologues with z, $z + n$, and $z + 2n$ carbon atoms. For the case of unevenly spaced homologues of z, $z + x$, and $z + y$ carbon atoms, the expression has been generalized [18] to:

$$\frac{t_{R,3} - t_{MM}}{t_{R,2} - t_{MM}} = \left(\frac{t_{R,2} - t_{MM}}{t_{R,1} - t_{MM}} \right)^{\frac{x}{y}} \quad (5)$$

and from this expression, the mathematical hold-up time may be derived. Other methods calculate adjusted retention times directly [17,18,20]. The value of t_{MM} is then obtained by subtraction.

A second group of methods based on the linearity principle uses four or more homologues (statistical and iterative methods) to compensate for the natural variation of the individual experimental retention times. Perhaps the most popular among this type of methods is that of Guardino *et al.* [21], which starts with an initial estimate of t_{MM} that must be lower than the true hold-up time. A similar method producing equivalent results [16,22] may be used with any initial estimate of t_{MM}.

The value of the hold-up time deduced with these linearity methods depends on the homologues used, confirming that the methods may be questionable. This dependence has been explained [19] because the "semilog plot" is not a straight line but rather a curve, at least up to 17 carbon atoms. The curvature depends on the actual value of t_M^g used to adjust retention times. It has been shown for the case of the n-alkanes that the value of t_{MM} obtained by any of the "linearity methods", will approximate the true value of t_M^g if early eluting homologues are used, and will gradually depart from that value as heavier homologues are employed [19]. This assertion is not necessarily true for the case of other homologous series [23].

4.1.2.2 Nonlinearity methods

It has been suggested that the chromatographic behavior of the n-alkanes is best described by an equation that does not imply linearity of the "semilog plot" [15]. A selection from 25 expressions, including the one that supports the "linearity principle" has brought about the conclusion that under a variety of column types and chromatographic conditions, the best description of the retention times of the n-alkanes is given by:

$$t_{R,z} = A + \exp\,(B + C\, z^{D}) \quad (6)$$

where z is the carbon number of the n-alkane [15,16]. From this expression, the hold-up time is obtained for a value of z equals zero. A minimum of five n-alkanes including methane must be injected to deduce the parameters A, B, C, and D by a nonlinear regression procedure. The value of the calculated hold-up time has been checked against the actual retention times of H_2, He, N_2, O_2, Ne, and Ar, and against values deduced by linearity methods [16], arriving at the conclusion that this procedure reproduces the retention of an unretained substance better than other methods. Thermodynamic, statistical, and structural reasons to support the nonlinear chromatographic behavior of n-alkanes have been presented [23,24]. The relation of the parameters A, B, and C of eq. 6 with different chromatographic variables has also been published [25]. Parameter D, which explains the lack of linearity of the "semilog plot", is a property of the methylene group, and has no relation to any chromatographic variable.

In the absence of further studies, the nonlinear methods are the recommended procedures to evaluate t_{MM} for gas chromatography.

4.2 Liquid chromatography

Numerous papers and reviews [26–30] have discussed the different methods used to measure the hold-up volume in liquid chromatography. A short presentation of these methods follows.

Similarly to the situation in gas chromatography, there are no ideal unretained substances that could be conveniently used for a direct measurement of the hold-up volume (time) of the chromato-

graphic column. All test compounds suggested in direct methods are either slightly retained by the stationary phase or slightly excluded from certain parts of the mobile phase in the column. The same statement is valid for components of a mixed mobile phase if any of the components is used as an unretained test compound. To evaluate the validity and precision of all techniques described below, one has to take into consideration the balance between the adsorption/exclusion phenomena mentioned above.

4.2.1 Direct methods

4.2.1.1 Methods using inorganic and organic compounds

Inorganic salts, particularly sodium nitrate and sodium nitrite, and some organic compounds, such as uracil, have been used as "markers" for hold-up volume determination. It has been shown [30] that hold-up volumes measured using inorganic salts depend on experimental condition (mobile-phase composition, ionic strength, and sample size) and on the ionic volume of the salt. This has been interpreted as sample exclusion (due to either electrostatic or steric effects) from the pores of the packing material, which prevents the analyte from fully exploring the intraparticle volume [31]. Consequently, the hold-up volume measured using inorganic salts can vary between the total volume of the mobile phase in the column and a value close to the interparticle (exclusion) volume, depending on experimental conditions.

The use of organic ions or highly polar analytes for hold-up volume determination should be avoided because most of them are retained to some extent, particularly in reversed-phase LC (RPLC) with mobile phases containing either moderate or high percentages of water.

4.2.2 Indirect methods

4.2.2.1 Method of column weight

In this technique, the column is filled and weighed successively with a light solvent of density δ_1 (e.g., methanol), and a heavy solvent of density δ_2 (e.g., carbon tetrachloride). The total volume of the mobile phase in the column V_t can be calculated by:

$$V_t = (m_2 - m_1)/(\delta_2 - \delta_1) \tag{7}$$

where m_1 and m_2 are the weights of the column filled with the light and the heavy solvent, respectively. It has been suggested that V_t may be the only measure of the hold-up volume V_M with a physical meaning, because it represents the volume within the column accessible to the two solvents and an analyte molecule of a size comparable to that of the solvent molecules. However, with solvents that are strongly adsorbed on the sorbent surface or that solvate the stationary phase to a significant extent (thus increasing the effective volume of the stationary phase at the expense of the mobile phase), this method may give negative k values for unretained compounds [32]. For the same reason, when this method is used for hold-up volume determination in RPLC the plots of log k vs. n_C (the number of carbon atoms on the alkyl moiety for each member of the homologous series) and log k vs. $1/T$ (inverse of the column temperature) are nonlinear for some solvents (particularly for methanol/water and acetonitrile/water mixtures) [32].

4.2.2.2 Methods using homologous series

Several methods to calculate the hold-up volume rely on the use of homologous series of analytes. These methods are based on a linear relationship between log V_R' vs. n_C if Gibbs energy of sorption of each analyte on the stationary phase increases by a constant amount from one homologue to the next in the series.

In one approach, the hold-up volume is calculated by self-consistent fitting of the equation:

$$\log V_R' = \log (V_R - V_M) = \mathrm{a}\, n_C + \mathrm{b} \tag{8}$$

Using different sets of probes, the value corresponding to the highest correlation coefficient is taken as the correct V_M. In another approach, the retention volume of each homologue is plotted against the retention volume of the previous one in the series. The hold-up volume can then be calculated from

the intercept of this straight line. The size (n_C) of the homologues used in the determination of V_M is of critical importance to obtain self-consistent data. Some authors [32] recommend the use of "convergent" conditions (i.e., chemical series) and chromatographic mobile phases for which the calculated hold-up volume does not change significantly (less than 5% being acceptable) with the n_C value and the total number of homologues chromatographed.

This method leads to values of the hold-up volume that are systematically smaller than V_t, because the exclusion effect for each homologue increases systematically with the increase of the length of the alkyl chain. Considering what has been said about the validity of eq. 8 in GC, further study of its application in LC is desirable.

4.2.2.3 Methods using isotopically labeled compounds

Isotopically labeled counterparts of the mobile-phase components have been used as hold-up volume markers in LC, particularly in RPLC studies. Usually, in these experiments, a deuterated component of the mobile phase (D_2O or a D-labeled modifier) is employed as the sample, and a refractive index monitor or mass spectrometer is used as the detector. Radioactively labeled samples and radioactivity detectors have also been employed in some cases. In these methods, the following assumptions are made: 1) The labeled compound used has the same chromatographic behavior as that of the nonlabeled counterpart in the mobile phase, and therefore it should explore the same column volume as this component of the mobile phase. 2) There is no difference in retention volume between labeled and nonlabeled compounds due to the differences in molecular size. 3) There is no isotopic exchange between the labeled analyte and some components of the stationary phase. Two procedures have been proposed: the injection of components of the mobile phase labeled with isotopes (deuterium or radioactive isotopes) or the injection of pure D_2O.

When a sample containing D_2O (either pure or dissolved in the mobile phase) is injected in an LC system fitted with a refractive index detector, two peaks can be expected: one peak corresponds to deuterium oxide and the other to the so-called system peak. The deuterium oxide peak might in fact correspond mostly or even solely to the species DHO, because isotopic exchange with water could take place in the mobile phase. Herein, for the sake of simplicity, this peak will be referred to as D_2O, the peak of the deuterium oxide or an equivalent expression, regardless of its nature. The system peak appears because in RPLC a preferential adsorption of the organic modifier on the stationary phase takes place, whereas less adsorption of water occurs. Injection of a sample of D_2O, even diluted in the mobile phase, can disturb the adsorption equilibrium and generate a transient enrichment of the mobile phase in one of its components that can be detected (by a refractometer detector) as a system peak. This system peak can be identified because its position is identical to that obtained by injecting H_2O-rich mobile phase as analyte. The other peak, corresponding to deuterium oxide, has been proposed [33] as a marker of the hold-up volume in RPLC.

It has been observed [33] that the retention of D_2O varies significantly with the modifier percentage of the mobile phase. The retention volume of D_2O tends toward V_t both at high and low concentration of modifier in the mobile phase, whereas values smaller than V_t are obtained for the retention volume of D_2O at intermediate compositions. It has also been observed [33] that the discrepancy between the retention volume of D_2O and V_t depends on the hydrophilic character of the modifier, the highest being for tetrahydrofuran. This variation in the retention volume of D_2O with the mobile-phase composition is related to the volume of modifier immobilized on the stationary phase. Since isotopic exchange between D_2O and methanol molecules immobilized in the RP packing takes place, some additional retention of deuterium oxide can be expected in those experiments where RPLC is used with water-methanol mobile phases. Isotopic exchange of injected D_2O with available silanol groups can also contribute to some retention of this substance in RP columns.

4.2.2.4 Minor disturbance method

In this approach, usually a small volume of the mobile phase with a slightly altered concentration in one of its components is injected in the column. In the simplest case of reversed-phase chromatography with

a two-component mobile phase and if the effluent of the column is monitored with a suitable detector (generally a refractometer), only one peak due to the disturbance of the mobile phase adsorbed on the stationary phase is observed. The retention volume corresponding to the maximum of this peak is related to V_t through the slope of the partition isotherm of the organic modifier on the stationary phase at the mobile-phase composition at which the experiment is carried out [34,35], thus, the retention volume of this peak depends on the eluent composition. Only for those mobile-phase compositions at which the slope of the partition isotherm approaches a value of unity, the retention volume of the disturbance peak approaches the value of the total volume of mobile phase in the column (V_t).

Due to these results, the minor disturbance method has been generally considered to be unreliable in LC. However, it has been shown [30] that using Gibbs model of adsorption a new equation to calculate the hold-up volume can be derived. This equation, which uses the retention volume of the disturbance peak at different mobile-phase compositions, gives values of the hold-up volume which are independent of column temperature and mobile-phase composition, and agree, within 5%, with the value of V_t of the column.

4.3 SUPERCRITICAL-FLUID CHROMATOGRAPHY

Hold-up times have been measured or calculated for capillary SFC with carbon dioxide as mobile phase [36,37]. Hold-up times were measured directly (methane injection), or calculated according to models based on the linearity principle of the semilog plot of the retention factor vs. carbon number [36], or based on polynomials that include mobile-phase density and temperature terms [37]. The validity of the different evaluation procedures was checked by comparison of calculated hold-up times, with those deduced from methane injections. A maximum was found for t_M when plotted vs. mobile-phase density. Bearing in mind that methane is not recommended as a hold-up time indicator in GC [15,16], and that under SFC conditions, elution order is strongly dependent on both temperature and mobile-phase density, it is felt that further work should be carried out before a conclusion may be drawn about hold-up times/volumes in SFC.

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Retention parameters in chromatography

(IUPAC Recommendations 2001)

Part B. Retention parameters in gas chromatograpy

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CONTENTS

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1. INTRODUCTION

This paper reexamines the terms for retention parameters in gas chromatography described previously in sections 3.6 and 3.7 of "Nomenclature for chromatography" [1] with the aims of (i) emphasizing the physical meaning of the terms and (ii) specifying the temperatures and pressures for the terms for gas volumes and flow rates.

2. IDEALIZED GAS CHROMATOGRAPHIC SYSTEM

When developing the theory of a process and defining the basic phenomena to be observed and the parameters to be measured during the process, one has to start by considering an idealized system. Thus, the ideal gas chromatographic system consists of an ideal gas mobile phase and an idealized liquid or solid stationary phase. This means that the mobile phase behaves and compresses like an ideal gas, thus meeting the requirements of the basic equation of state for gases

$$p \cdot V / T = n \cdot R \quad (1)$$

whereas the stationary phase is totally incompressible. Moreover, the carrier gas is assumed to be insoluble in the stationary liquid phase and unretained by the solid adsorbent. It is further assumed that the molecules of the carrier gas do not interact with analyte molecules and do not compete with the interactions of the latter with the stationary phase. Needless to say, it is assumed that the chromatographic column has a constant diameter and the stationary phase is evenly packed in the column.

In this case, the retention of the solute (analyte) will depend only on its interaction with the stationary phase. Differences in the interactions of solutes with the stationary phase provide the basis for all types of chromatographic separations. In linear equilibrium chromatography it is further assumed that the equilibrium distribution of the solutes between the two phases of the chromatographic system is instantly established at every position in the moving chromatographic bands, so that only thermody-

namic, and not kinetic phenomena have to be considered when describing the idealized chromatographic system.

The simplest way to characterize quantitatively the interaction of a solute with the stationary phase is by measuring the rate of migration of the solute along the column and comparing this rate with the flow rate of the carrier gas (or, which is the same, with the migration rate of a hypothetical unretained test compound). Flow rates and migration rates are the volumes of the mobile phase which pass any column cross-section in a specified period of time.

3. CORRECTION OF MEASURED RETENTION PARAMETERS IN GC

What can be measured in a typical gas chromatographic experiment are the *inlet pressure* (p_i), *outlet pressure* (p_o), *ambient pressure* (p_a), *column temperature* (T_c), *ambient temperature* (T_a), and the *volume* (V) of mobile phase that leaves the column outlet in defined periods of *time* (t), and hence, the carrier gas *flow rate* (F).

Since the volume of a gas varies markedly with temperature and pressure, these two parameters have always to be precisely specified for any measured volume. If there is no restrictor installed at the column outlet and if the pressure drop within the detector is negligibly small, the column outlet pressure equals the ambient pressure, $p_o = p_a$. (In the case of linked gas chromatography–mass spectrometry, the outlet column pressure is often close to the vacuum in the mass spectrometer.) However, even in the case that the column temperature does not differ from the ambient temperature, the gas volume measured at the column outlet does not characterize the elution process inside the column, since the gas pressure in the column will differ from that outside. The pressure gradually drops from the value at the column inlet to that at the outlet. Consequently, the mobile-phase gas expands as it moves along the column. Accordingly, gas volume flow rates, when measured at different cross-sectional positions along the column, would show a gradual increase. These changes in the gas pressure, volume, and flow rate are not linear within the column. The changes are slow at the beginning, but become more significant toward the column outlet. In order to characterize the chromatographic process in the column by using elution volumes that were measured at ambient temperature and pressure at the column outlet, these values of the gas volume have to be corrected for the temperature and compressibility of the gas. In other words, the volumes have to be calculated for the column temperature and the appropriate gas pressure in the column, namely, *the average gas pressure over the column length* ($\bar{p}$).

In order to derive a formula for calculating the average gas pressure from the pressure at the column inlet and outlet, three important statements have to be accepted:

(i) The averaged gas pressure value should meet the requirements of the basic ideal gas eq. 1.
(ii) The volume of the mobile phase (carrier gas) that is contained in the column at any instant, irrespective of the nature of the gas, its viscosity, pressure, flow rate, pressure drop, or temperature, must be equal to the total geometrical volume of space that is available for the mobile phase (V_t), sometimes called void volume.
(iii) An unretained solute must elute from the column at the void volume of the mobile phase in the column.

In gas chromatography, the volume of the mobile phase which is required for the elution of an unretained compound is measured experimentally *under ambient conditions* (V_M^a). It differs from the volume of the mobile phase in the column because the ambient pressure and temperature differ from the actual conditions within the column. (Extra-column volumes comprising the volumes of the sample injector, the detector, and connecting lines are assumed to be negligibly small and are not considered here.) According to (i)–(iii), the following relationships must be valid:

$$\bar{p} \cdot V_t / T_c = p_o \cdot V_M^a / T_a \quad (2)$$

$$V_t = V_M^a \cdot (T_c / T_a) \cdot (p_o / \bar{p}) \tag{3}$$

which should allow the calculation of the actual gas volume involved in the elution of the unretained compound V_t $(\bar{p}, T_c)$ from the measured gas volume $V_M^a(p_o, T_a)$.

In eq. 3, the temperature correction factor, (T_c/T_a) is usually larger than 1, since gas chromatography is normally conducted at elevated column temperatures. The term $p_o / \bar{p} = j$, called the "mobile-phase compressibility correction factor", is always smaller than 1 and shows to what extent the mobile phase was compressed while in the column, compared to the situation at the column outlet, where it was exposed to a lower pressure p_o. Symbol j was chosen for this correction factor (eq. 4), to commemorate James and Martin, who first calculated and introduced this term as early as 1952 [2]:

$$j_3^2 = \frac{3}{2} \cdot \frac{(p_i / p_o)^2 - 1}{(p_i / p_o)^3 - 1} = p_o / \bar{p} \tag{4}$$

A detailed derivation of eq. 4 for the compressibility correction factor, that provides the averaging of ideal carrier gas pressure over the column length, can be found in the literature [3,4]. In the second reference [4], it was also shown that, alternatively, averaging of the varying pressure to which the analyte is exposed can be done over the time that the solute resides in the column. In this case, however, another j factor must be used, namely j_4^3. Similarly, by using two different j factors, j_2^1 and j_3^2, the flow rate F of the carrier gas can be averaged either over the column length or over the time of residence of a sample in the column. These various j values are presented in Table 1, according to the general expression by Everett [5] for different mobile-phase compressibility correction factors:

$$j_n^m = \frac{n}{m} \cdot \frac{(p_i / p_o)^m - 1}{(p_i / p_o)^n - 1} \tag{5}$$

where $n = m + 1$.

Table 1 Average values of mobile-phase gas pressure and flow rates in a chromatographic column (at $T_c = T_a$) [4].

Averaging over the column length ($x = L$)		Averaging over the time ($t = t_M$) of residence of an unretained compound in the column	
Average pressure	Average flow rate	Average pressure	Average flow rate
$\bar{p}_x = p_o / j_3^2$	$\bar{F}_x = F_o \cdot j_2^1$	$\bar{p}_t = p_o / j_4^3$	$\bar{F}_t = F_o \cdot j_3^2$

From eq. 5, $m = 1$, $n = 2$ when the flow rate is averaged over the column length; $m = 2$, $n = 3$ when the pressure is averaged over the column length or the flow rate is averaged over time; $m = 3$, $n = 4$ when the pressure is averaged over the time that a solute resides in the column. For ideal as well as traditional gas chromatography (i.e., at moderate pressures and pressure drops), the "classical" James and Martin's factor j_3^2 should be applied for calculating corrected retention parameters. Equations that incorporate j_4^3 and even j_5^4 have been suggested for chromatography with nonideal carrier gases, especially at elevated pressure values and flow rates [6,7].

The James and Martin compressibility correction factor j_3^2 can thus convert all volumes that were measured under ambient pressure conditions at the column outlet into the corresponding volumes *under the pressure averaged over the column length*:

Corrected gas hold-up volume $V_M^o = V_M \cdot j_3^2$

Corrected retention volume $V_R^o = V_R \cdot j_3^2$

Net retention volume $V_N = (V_R - V_M) \cdot j_3^2$

After being corrected for temperature and pressure* the hold-up volume becomes identical to the total volume of the mobile phase in the column, i.e., the geometrical void volume ($V_M^o = V_t$). Similarly, the corrected solute retention volume becomes expressed in the same volume units of the mobile phase under the actual conditions of chromatography. Therefore, the corrected retention and hold-up volumes can be simply related to the retention factor (k) as:

$$V_R^o = (1+k) \cdot V_M^o \tag{6}$$

4. THERMODYNAMIC NATURE OF CORRECTED RETENTION PARAMETERS

It is necessary to emphasize that the corrected (for temperature and pressure) retention volumes of analytes, as solute-characteristic retention parameters, are *independent of the pressure* of the carrier gas (both inside and outside the column). In full agreement with the initial model of ideal gas chromatography, they are entirely determined by the intensity of interaction of the solutes with the stationary phase, not with the mobile phase, and therefore acquire thermodynamic meaning. The thermodynamic significance is especially evident for the *specific retention volumes* (V_W, V_V, V_A), which are obtained by normalizing the *net retention volumes* for, respectively, the weight, volume of the stationary phase in the column, or the surface area of the solid packing in the column. Specific retention volumes are directly proportional to (or, in the case of V_V, equal to) the corresponding *distribution coefficients K* of the solutes between the stationary and mobile phases contained in the column at the column temperature [4].

While being independent of the pressure conditions during the chromatographic experiment, the corrected and specific retention volumes are still strongly dependent on the column temperature. If the Gibbs energy of transfer of the solute from the ideal gas phase into the liquid phase is ΔG, the temperature dependence of the distribution coefficients (and retention volumes) follows from the fundamental thermodynamic eq. 7:

$$\Delta G = -RT_c \cdot \ln K = -RT_c \cdot \ln V_V \tag{7}$$

Accordingly, ln K and ln V_V should vary linearly with the inverse column temperature:

$$\ln V_V = -(\Delta G / R) \cdot 1 / T_c = -(\Delta H / RT_c + \Delta S / R) \tag{8}$$

which, indeed, was repeatedly shown to be the case (within temperature ranges where ΔH and ΔS remain constant). It is natural that corrected for column-averaged pressure and real column temperature values of retention volumes should be taken for plotting against inverted temperature according to eq. 8. It must be mentioned in this connection that many textbooks, as well as the IUPAC document [1], misleadingly introduce a value called "specific retention volume" at 0 °C, which is obtained by multiplying the specific retention volumes by the ratio ($273.15/T_c$). This term attempts to provide a base for comparing retention parameters at a standard temperature of 0 °C. As shown in the works of Davankov and Parcher [3,8,9], such a "recalculation to standard temperature" will significantly distort the actual relationship between retention volumes measured at different temperatures, and it should never be

*If the initial volume or flow rate was measured using a so-called soap bubble flow meter, a third correction factor, $(1 - p_w/p_o)$, must be introduced, where p_w is the partial pressure of water vapor at ambient temperature, to take into account the dilution of the effluent gas with water vapor.

applied. It is the volume, not the mass, of the mobile phase that matters in the chromatographic elution process. Therefore, retention volumes can represent the process thermodynamics only under the condition that they relate to the actual situation in the column with respect to temperature and pressure.

Equally misleading are the recommended terms in ref. 1, "corrected retention time" and "net retention time", which incorporate the *mobile-phase compressibility correction factor (j),* as explained in ref. 8. Time has nothing to do with gas compressibility. This error was caused by the lack of a precise definition of the compressibility correction factor, which would clearly emphasize its simple and sole physical meaning, that is $p_o / \bar{p} = j$.

To take into account the physical sense of the j factors, paying attention to the averaging modes and actual pressures and temperatures of the carrier gas, the definitions of terms dealing with the mobile-phase flow rate must also be provided with relevant additional information, such as:

The mobile-phase flow rate at the column cross section where the pressure is equal to the pressure averaged over the column length: $F_{\bar{p}} = F_a \cdot j_3^2 \cdot (T_c / T_a)$.

The Appendix section Terms and Definitions redefines only those terms (as compared to sections 3.6 and 3.7 in the original "Nomenclature for Chromatography" [1]) which require changes, as a consequence of the revised physical meaning of the measured or calculated chromatography conditions and solute retention parameters that are outlined above.

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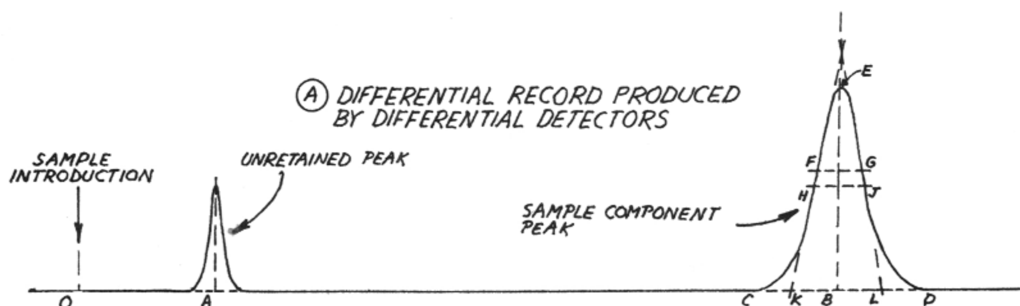
Appendix

A1. TERMS AND DEFINITIONS THAT HAVE BEEN INTRODUCED OR MODIFIED

In the following list of terms, the numbering follows that in the IUPAC "Nomenclature for Chromatography", *Pure Appl. Chem.* **65**, 819–872 (1993). The figure mentioned below is reproduced in part from that paper.



1.1 General terms

1.1.01.1 Chromatographic phase system
A system formed by at least two immiscible phases in contact with each other, one of which is continuously moving relative to the other. Normally, one of the phases remains stationary and is that part of the chromatographic phase system responsible for the retention of the analytes, whereas the moving part is responsible for their transportation.

1.1.01.2 Chromatographic process
The dynamic distribution that takes place between phases when an analyte (or a mixture of analytes) is carried along the stationary phase by the movement of the mobile phase.

1.1.01.3 Sample introduction
The act of introducing the sample into the apparatus that contains the chromatographic phase system, to obtain a chromatogram. The term "sample injection" is used to indicate sample introduction into the mobile phase through an injector.

1.1.01.4 Initiation time
The moment at which the chromatographic process starts (when the sample starts to move along the chromatographic phase system). Normally, in column chromatography the moment when the sample, carried by the mobile phase, enters the column.

3.6 The mobile phase

3.6.03 Mobile-phase compressibility correction factor (j_3^2)
The ratio of the gas pressure at the column outlet (p_o) to the gas pressure in the column *averaged over the column length* ($\bar{p}$):

$$j_3^2 = p_o / \bar{p}$$

James and Martin presented the following equation for calculating the factor j_3^2 for an ideal carrier gas from its inlet pressure (p_i) and outlet pressure (p_o):

$$j_3^2 = \frac{3}{2} \cdot \frac{(p_i / p_o)^2 - 1}{(p_i / p_o)^3 - 1} = p_o / \bar{p}$$

$$\text{or} \quad j_3^2 = \frac{3}{2} \cdot \frac{P^2 - 1}{P^3 - 1} = p_o / \bar{p}$$

where $P = p_i / p_o$ is *relative pressure*, the ratio of the inlet and outlet pressures.

Note: The factor j_3^2 is always smaller than 1. It shows the extent to which the mobile phase is compressed when residing in the column, as compared to the situation at the column outlet, where it is exposed to a smaller pressure, p_o, usually ambient pressure, p_a.

3.6.04 Flow rate

The volume of mobile phase passing through the column in unit time.

Since, in gas chromatography, the mobile phase is a compressible gas, the corresponding pressure and temperature conditions must be always specified.

3.6.04.1 Carrier gas flow rate at ambient temperature and ambient pressure (F_a)
Carrier gas flow rate measured at the column outlet under ambient temperature and ambient pressure.

Note: Gas flow rate is often measured at the column outlet at ambient pressure (p_a) and ambient temperature (T_a, in *K*) using a water-containing flow meter (the so-called soap bubble meter); this value is indicated with the symbol *F*. Since the carrier gas becomes diluted with water vapor in the soap bubble meter, the measured flow rate *F* must be corrected (reduced):

$$F_a = F \cdot (1 - p_w / p_a)$$

where p_w is the partial pressure of saturated water vapor at ambient temperature.

3.6.04.2 Carrier gas flow rate at column temperature and ambient pressure (F_c)
Carrier gas flow rate at the column outlet corrected for column temperature and ambient pressure.

$$F_c = F_a \cdot (T_c / T_a) = F \cdot (1 - p_w / p_a) \cdot (T_c / T_a)$$

3.6.04.3 Corrected carrier gas flow rate (carrier gas flow rate in the column cross-section with pressure equal to that averaged over column length) ($F_c^o = F_{\bar{p}}$)
Carrier gas flow rate corrected for column temperature and pressure averaged over column length.

$$F_c^o = F_{\bar{p}} = F_c \cdot j_3^2 = F_a \cdot (T_c / T_a) \cdot j_3^2 = F \cdot (1 - p_w / p_a) \cdot (T_c / T_a) \cdot j_3^2$$

3.6.04.4 Carrier gas flow rate at column temperature averaged over column length ($\bar{F}_x$)
Carrier gas flow rate corrected for column temperature and averaged over column length.

$$\bar{F}_x = F_c \cdot j_2^1 = F_a \cdot (T_c / T_a) \cdot j_2^1 = F \cdot (1 - p_w / p_a) \cdot (T_c / T_a) \cdot j_2^1$$

$$\text{where} \quad j_2^1 = 2 \cdot \frac{(p_i / p_o) - 1}{(p_i / p_o)^2 - 1} = 2 \cdot \frac{P - 1}{P^2 - 1}$$

3.7 Retention parameters in column chromatography

3.7.03.1 Hold-up volume (at ambient temperature and pressure) (V_M^a)
The volume of the mobile phase required to elute the unretained compound from the chromatographic column and measured at ambient pressure and temperature.

$V_M^a = t_M \cdot F_a$

Note: In gas chromatography and supercritical-fluid chromatography, this value does not represent the mobile-phase volume in the column.

3.7.03.2 Hold-up volume at column temperature and ambient pressure (V_M)
The volume of the mobile phase required to elute the unretained compound from the chromatographic column and reported at column temperature and ambient pressure.

$V_M = t_M \cdot F_c$

Note: In gas chromatography and supercritical-fluid chromatography, this value does not represent the mobile-phase volume in the column.

3.7.03.3 Gross hold-up volume (V_M^g)
The volume of the mobile phase that leaves the column from the moment of sample introduction to the detection of the peak maximum of an unretained compound and reported at column temperature and ambient pressure. It also includes the *extra-column volumes.*

$V_M^g = t_M^g \cdot F_c$

Note: This corresponds to the previous definition of hold-up volume (NC 3.7.03).

3.7.03.4 Hold-up time (t_M)
Time required for the mobile phase to pass through the chromatographic column. (Residence time of an unretained compound in the chromatographic column.)

Note: The hold-up time corresponds to the distance OA in Fig. 1, if the extra-column volumes have been eliminated. Sometimes it is also referred to as t_0, but this symbol should be reserved to denote the retention time of an unretained (totally excluded) compound in exclusion chromatography. Its use in elution chromatography is discouraged.

Note: Mathematical hold-up time (t_{MM}). The hold-up time deduced by a mathematical treatment of retention data of the chromatogram. The methods available at present produce a value that will normally approximate the "gross hold-up time".

3.7.03.5 Gross hold-up time (t_M^g)
Time elapsed from the sample introduction to the detection of the peak maximum of an unretained compound. It includes the time required for the mobile phase to pass through the *extra-column volumes.*

3.7.04 Corrected carrier gas hold-up volume (at column temperature and pressure averaged over column length) (V_M^o)
The volume of the mobile phase required to elute the unretained compound from the chromatographic column and reported at column temperature and pressure averaged over column length:

$V_M^o = V_M \cdot j_3^2 = t_M \cdot F_c \cdot j_3^2 = t_M \cdot F_c^o$

Note: The corrected gas hold-up volume equals the geometrical void volume of the column that is available to the mobile phase, $V_M^o = V_o$.

3.7.05.1 Retention volume at ambient temperature and pressure (V_R^a)
The volume of the mobile phase required to elute the compound of interest from the chromatographic column and measured at ambient pressure and temperature.

$V_R^a = t_R \cdot F_a$

3.7.05.2 Retention volume at column temperature and ambient pressure (V_R)
The volume of the mobile phase required to elute the compound of interest from the chromatographic column and reported at column temperature and ambient pressure.

$$V_R = t_R \cdot F_c$$

3.7.05.3 Gross retention volume (at column temperature and ambient pressure) (V_R^g)
The volume of the mobile phase that leaves the column from the moment of sample introduction to the detection of the peak maximum of the analyte and reported at column temperature and ambient pressure. It also includes the *extra-column volumes.*

$$V_R^g = t_R^g \cdot F_c$$

Note: This corresponds to the previous definition of *total retention volume* (NC 3.7.05).

3.7.05.4 Retention time (t_R)
Time required for the analyte to pass through the chromatographic column. (Residence time of the analyte in the chromatographic column.)

Note: The retention time corresponds to the distance OB in Fig. 1A, if the extra-column volumes have been eliminated.

3.7.05.5 Gross retention time, (t_R^g)
Time elapsed from the moment the time count is initiated, to the detection of the maximum of the peak of the analyte in the mobile phase. It includes the time required for the analyte to pass through the *extra-column volumes.*

3.7.07.1 Adjusted retention volume (at column temperature and ambient pressure) (V_R')
The retention volume minus the hold-up volume:

$$V_R' = V_R - V_M = (t_R - t_M) \cdot F_c$$

3.7.07.2 Adjusted retention time (t_R')
The retention time minus the hold-up time.

$$t_R' = t_R - t_M$$

Note: The adjusted retention time corresponds to the distance AB in Fig. 1A.

3.7.08 Corrected retention volume (at column temperature and pressure averaged over column length) (V_R°)
The volume of the mobile phase required to elute the compound of interest from the chromatographic column and reported at column temperature and pressure averaged over column length.

$$V_R^o = V_R \cdot j_3^2 = t_R \cdot F_c \cdot j_3^2$$

3.7.09 Net retention volume (at column temperature and pressure averaged over column length) (V_N)

The adjusted retention volume reported at column temperature and pressure averaged over column length:

$$V_N = V_R^o - V_M^o = V_R' \cdot j_3^2 = (t_R - t_M) \cdot F_c \cdot j_3^2 = t_R' \cdot F_c^o$$

3.7.11 Specific retention volume (at column temperature and pressure averaged over column length) (V_W, V_V, V_A)

The net retention volume per unit weight of stationary phase (V_W), unit volume of the stationary liquid phase (V_V), or unit surface area of the solid column packing (V_A).

A2. TERMS TO BE DROPPED

These terms are regarded as obsolete, and their use is strongly discouraged.

3.7.05 Total retention volume (time)

These terms are confusing in that they seem to include the extra-column volume (or the time required for the mobile phase to pass through the extra-column volumes), which they should not. Usage of the terms is strongly discouraged.

3.7.08 Corrected retention time ($t_R°$)

The total retention time multiplied by the compressibility correction factor (j). This term erroneously includes the mobile-phase compressibility correction factor (j), as explained in Part B. Usage of the term is strongly discouraged.

3.7.09 Net retention time (t_N)

This term erroneously includes the mobile-phase compressibility correction factor (j), as explained in Part B. Usage of the term is strongly discouraged.

3.7.11.2 Specific retention volume at 0 °C

By multiplying the specific retention volume with the ratio ($273.15/T_c$), this term attempts to provide a base for comparing retention parameters at a standard temperature of 0 °C. As shown in Part B, such a "recalculation to standard temperature" will significantly distort the actual relationship between the retention volumes measured at different temperatures and should not be applied. The use of this expression is strongly discouraged.

A3. LIST OF TERMS

Terms that are new or have been redefined:

Adjusted retention time	3.7.07.2
Adjusted retention volume	
(at column temperature and ambient pressure)	3.7.07.1
Carrier gas flow rate	
at ambient temperature and ambient pressure	3.6.04.1
at column temperature and ambient pressure	3.6.04.2
at column temperature averaged over column length	3.6.04.4
Chromatographic phase system	1.1.01.1
Chromatographic process	1.1.01.2
Corrected carrier gas flow rate	3.6.04.3
Corrected carrier gas hold-up volume	
(at column temperature and pressure averaged over column length)	3.7.04
Corrected retention volume	
(at column temperature and pressure averaged over column length)	3.7.08
Flow rate	3.6.04
Gross hold-up time	3.7.03.5
Gross hold-up volume	3.7.03.3
Gross retention time	3.7.05.5
Gross retention volume (at column temperature and ambient pressure)	3.7.05.3
Hold-up time	3.7.03.4
Hold-up volume at ambient temperature and pressure	3.7.03.1
Hold-up volume at column temperature and ambient pressure	3.7.03.2
Initiation time	1.1.01.4
Mobile-phase compressibility correction factor	3.6.03
Net retention volume	
(at column temperature and pressure averaged over column length)	3.7.09

Retention time 3.7.05.4
Retention volume at ambient temperature and pressure 3.7.05.1
Retention volume at column temperature and ambient pressure 3.7.05.2
Sample injection 1.1.01.3
Sample introduction 1.1.01.3
Specific retention volume
(at column temperature and pressure averaged over column length) 3.7.11

A.4 LIST OF SYMBOLS

Symbols that are new or have been redefined:

Symbol	Meaning	Section
F_a	Carrier gas flow rate at ambient temperature and ambient pressure	3.6.04.1
F_c	Carrier gas flow rate at column temperature and ambient pressure	3.6.04.2
F_c^{o}	Corrected carrier gas flow rate	3.6.04.3
$F_{\bar{p}}$	Corrected carrier gas flow rate	3.6.04.3
$\bar{F}_x$	Carrier gas flow rate at column temperature averaged over column length	3.6.04.4
j_3^2	Mobile-phase compressibility correction factor	3.6.03
t_M	Hold-up time	3.7.03.4
t_{MM}	Mathematical hold-up time	3.7.03.4
t_M^g	Gross hold-up time	3.7.03.5
t_R	Retention time	3.7.05.4
t_R'	Adjusted retention time	3.7.07.2
t_R^g	Gross retention time	3.7.05.5
V_A	Specific retention volume per unit surface area of the solid column packing (at column temperature and pressure averaged over column length)	3.7.11
V_M^a	Hold-up volume at ambient temperature and pressure	3.7.03.1
V_M	Hold-up volume at column temperature and ambient pressure	3.7.03.2
V_M^g	Gross hold-up volume	3.7.03.3
V_M^o	Corrected carrier gas hold-up volume (at column temperature and pressure averaged over column length)	3.7.04
V_N	Net retention volume (at column temperature and pressure averaged over column length)	3.7.09
V_R^a	Retention volume (at ambient pressure and temperature)	3.7.05.1
V_R	Retention volume (at column temperature and ambient pressure)	3.7.05.2
V_R°	Corrected retention volume (at column temperature and pressure averaged over column length)	3.7.08
V_R'	Adjusted retention volume (at column temperature and ambient pressure)	3.7.07.1
V_R^g	Gross retention volume (at column temperature and ambient pressure)	3.7.05.3
V_V	Specific retention volume per unit volume of stationary liquid phase (at column temperature and pressure averaged over column length)	3.7.11
V_W	Specific retention volume per unit weight of stationary phase (at column temperature and pressure averaged over column length)	3.7.11