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Effect of Sample Volume in Linear Preparative Chromatography: A More Rigorous Treatment

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Abstract

This paper describes the effect of sample volume on some basic chromatographic parameters for linear preparative chromatography: apparent plate height, base width, resolution, and purity. The expressions derived here are based upon the moment representation and the properties of linear systems. The commonly used definition for analytical resolution is shown to lead to several distinctive relationships of preparative resolution according to the value of the sample volume V_0 and the analytical profile standard deviations σ_1 and σ_2 for the two compounds. For each case the expression of the sample volume corresponding to a preparative resolution of one is then derived. This paper gives the way to calculate the purity and recovery ratios of each fraction when the analytical profiles are Gaussian. It is worth keeping in mind that in analytical chromatography the purity of a fraction is related to the resolution and the sample concentration ratio only, whereas in preparative chromatography, purity is also dependent on sample volume and analytical profile standard deviations. This is why resolution is not a concept as useful for preparative purposes as for analytical ones.

INTRODUCTION

Recently a number of theoretical studies have appeared on the effect of sample volume in preparative liquid chromatography. Generally, all

the given relationships implicitly assume the linearity of the chromatographic process. Furthermore, the preparative concepts are sometimes erroneously defined and the simplifying assumptions often not clearly understood. However, the topic was rigorously broached for preparative gas chromatography as early as 1961 by Pretorius et al. (*1*) and then by Reilley et al. (*2*, *3*).

Our present purpose is to discuss the useful results concerning the effect of sample volume on the main chromatographic parameters (base width, apparent plate height, resolution, and purity) in linear preparative chromatography. At first glance, one more paper on the subject could be considered as uninteresting. We feel that the parameter definitions and the validity of the relationships should be set more clearly. It must be noted that the rigorous relationships are not more intricate than the commonly used simplified ones.

APPARENT PLATE HEIGHT

If the chromatographic process is linear, the output profiles can be advantageously described by their first two moments, defined in Ref. *4*. By means of the moments, the analytical peak (impulse response characterizing the intrinsic behavior of the process) is defined by the retention volume V_R ($V_R = M_1$: first-order moment) and the standard deviation σ ($\sigma = \sqrt{M_2}$; M_2: second-order centered moment). The plate number N and the height equivalent to a theoretical plate H are in turn defined by

$$N = \frac{V_R^2}{\sigma^2} = \frac{(M_1)^2}{M_2}, \qquad H = L\frac{\sigma^2}{V_R^2} = L\frac{M_2}{(M_1)^2}$$

The first two moments of a V_0 width pulse-shaped injection profile (process input) are respectively $V_0/2$ and $V_0^2/12$.

As long as the chromatographic process behavior is linear, the moments of the column output profile (pulse injection response) can be derived from the preceding ones by mere addition (*5*):

$$M_1' = V_R + \frac{V_0}{2}, \qquad M_2' = \sigma^2 + \frac{V_0^2}{12}$$

The apparent plate number N' and the apparent plate height H' are defined by

$$N' = \frac{(M_1')^2}{M_2'} \qquad \text{and} \qquad H' = L\frac{M_2'}{(M_1')^2}$$

H' (or N') can be rigorously calculated in terms of H (or N), V_R, σ, and V_0:

$$\frac{H'}{H} = \frac{N}{N'} = \frac{V_R^2}{\sigma^2} \frac{\sigma^2 + V_0^2/12}{(V_R + V_0/2)^2}$$

If V_0 is small with regard to V_R, a simple relationship is obtained:

$$\frac{H'}{H} = \frac{N}{N'} = \left(1 + \frac{V_0^2/12}{\sigma^2}\right)\left(1 - \frac{V_0}{V_R}\right) \quad (1)$$

In particular, in analytical chromatography, if a loss of $\alpha\%$ in efficiency can be tolerated, a volume V_0 equal to $\sqrt{0.12}\alpha\sigma = \sqrt{0.12}\alpha(V_R/\sqrt{N})$ can be injected. If α is equal to 10%, V_0 is close to σ.

BASE WIDTH

The chromatographic parameters can be estimated from experimental profiles numerically or graphically. In the case of such a graphic calculation, the chromatographer often uses the base width W defined as the distance between the intersection points of the inflection tangents and the base line. W appears in the internationally accepted definition for resolution (*6*):

$$Rs = \frac{2(V_{R2} - V_{R1})}{W_1 + W_2}$$

From now on, the analytical peaks are supposed to be Gaussian. Then $W = 4\sigma$. The shape of a pulse response profile is dependent on the value of V_0 in comparison with σ, as is clearly shown in Ref. *7*:

If V_0 is larger than 4σ, the output profile exhibits a plateau related to the injected concentration C_0.

If V_0 is smaller than 4σ, the output profile does not show a plateau any more and its apex concentration is less than C_0. Consequently, if W' denotes the base width of a pulse response profile, the expression of W' in terms of V_0 depends on whether V_0 is larger than 4σ or not.

If V_0 is larger than 4σ, W' can be derived according to Fig. 1: the inflection tangent slope, given by the Gauss function, is equal to $1/\sqrt{2\pi}\sigma$. Hence

$$W' = V_0 + \sigma\sqrt{2\pi} \quad (2)$$

If V_0 is smaller than 4σ, Relation (2) is no longer valid. In that case, W' can be numerically calculated. An expression obtained by least squares

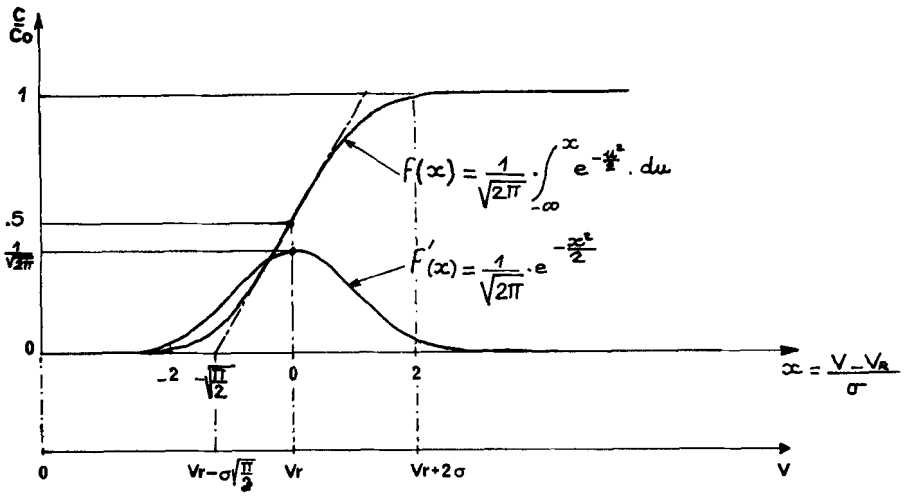

FIG. 1. Gaussian impulse response and the corresponding step response.

can be proposed:

$$W' = 4\sigma\left(1 + 0.04\frac{V_0^2}{\sigma^2}\right) \tag{3}$$

Reference 7 gives the following formula for this case:

$$W' = 4\sigma + V_0 \tag{4}$$

This formula leads to a 30% error for V_0 around 2.5σ. As a consequence, the relation between H' and H proposed in Ref. 7 is

$$\frac{H'}{H} = \left(\frac{W'}{W' - V_0}\right)^2$$

which has been derived from (4), and is a poor approximation. Moreover, the authors have put $H' = LW'^2/16V'^2_{max}$ which is not rigorous since the output profile, equal to the difference between two shifted Gauss function integrals, is not Gaussian. So the exact (and very simple) relationships to keep in mind are (1), (2), and (3).

RESOLUTION

With the help of Formulas (2) and (3), the effect of V_0 upon resolution can now be studied. In the following, three cases are considered with regard to the standard deviations σ_1 and σ_2 of both compounds. σ_2 is

assumed to be larger than (or equal to) σ_1, which is almost always the case. In our opinion, the following discussion has never been clearly developed up to now.

For $V_0 \leqslant 4\sigma_1$:

$$Rs' = \frac{V_{R2} - V_{R1}}{2(\sigma_1 + \sigma_2)\left(1 + 0.04\frac{V_0^2}{\sigma_1\sigma_2}\right)} \tag{5}$$

When V_0 goes down to zero, Expression (5) becomes the classical resolution formula between two Gaussian analytical peaks:

$$Rs = \frac{V_{R2} - V_{R1}}{2(\sigma_1 + \sigma_2)}$$

For $4\sigma_1 < V_0 < 4\sigma_2$:

$$Rs' = \frac{V_{R2} - V_{R1}}{\sqrt{\frac{\pi}{2}}\sigma_1 + 2\sigma_2 + \frac{V_0}{2} + 0.08\frac{V_0^2}{\sigma_2}} \tag{6}$$

Equation (6) is numerically more difficult to use. Furthermore, this case has a less wide scope, since σ_1 and σ_2 are of the same order of magnitude. This is why it will not be developed further here.

For $V_0 \geqslant 4\sigma_2$:

$$Rs' = \frac{V_{R2} - V_{R1}}{V_0 + \sqrt{\frac{\pi}{2}}(\sigma_1 + \sigma_2)} \tag{7}$$

MAXIMUM SAMPLE VOLUME

The maximum sample volume $V_{0_{lin}}$, that can be injected in linear preparative chromatography, can be calculated by putting $Rs' = 1$. This leads to the following.

If $V_{0_{lin}} \leqslant 4\sigma_1$:

$$V_{0_{lin}} = 5\sqrt{\sigma_1\sigma_2(Rs - 1)} \tag{8}$$

in which Rs is the analytical resolution.

If $V_{0_{lin}} \geqslant 4\sigma_2$:

$$V_{0_{lin}} = V_{R2} - V_{R1} - \sqrt{\frac{\pi}{2}}(\sigma_1 + \sigma_2) = 2(\sigma_1 + \sigma_2)\left(Rs - \sqrt{\frac{\pi}{8}}\right) \tag{9}$$

In practice, the conditions $V_{0_{1in}} \leqslant 4\sigma_1$ and $V_{0_{1in}} \geqslant 4\sigma_2$ are not convenient to use, since they can be met only after calculating $V_{0_{1in}}$ by means of (8) and also of (9). They can be replaced by conditions on the analytical resolution Rs. Numerical calculations have shown that for a ratio σ_2/σ_1 less than 2, the choice between relationships (8) and (9) can be taken according to the Rs value only. These calculations have also shown that relationships (8) and (9) allow $V_{0_{1in}}$ to be obtained with a precision better than 3%, even in the case $4\sigma_1 < V_{0_{1in}} < 4\sigma_2$. Finally $V_{0_{1in}}$ is given by the following.

For $1 < Rs < 1.3$:

$$V_{0_{1in}} = 5\sqrt{\sigma_1\sigma_2(Rs - 1)} \tag{8}$$

For $Rs \geqslant 1.3$:

$$V_{0_{1in}} = 2(\sigma_1 + \sigma_2)(Rs - 0.63) \tag{9}$$

Most often, these two cases are not distinguished in the literature (*8*, *9*), where the following single expression is to be seen:

$$V_{0_{1in}} = V_{R2} - V_{R1} - 2(\sigma_1 + \sigma_2) = 2(\sigma_1 + \sigma_2)(Rs - 1) \tag{10}$$

We personally used this expression in (4), (10), and (11). However, Coq et al. (*9*) are right to mention that the term $(\sigma_1 + \sigma_2)$ may become negligible in certain cases. The better known relationships (10) can therefore be kept, but it is worth keeping in mind that it comes from a different and stricter definition for resolution:

$$Rs' = \frac{V_{R2} - V_{R1}}{V_0 + 2(\sigma_1 + \sigma_2)}$$

PURITY

In the case of a binary mixture separated into two fractions 1 and 2 (let V_c be the elution volume at which the effluent is cut between the two fractions), the impurity ratios Ti_1 and Ti_2 can be defined as Pretorius et al. (*1*) properly did:

$$Ti_1 = \frac{Q_2}{Q_{0_1} - Q_1}, \qquad Ti_2 = \frac{Q_1}{Q_{0_2} - Q_2}$$

where Q_{0_1} and Q_{0_2} denote the injected amounts of each compound. Q_1 is the amount of Compound 1 contained in Fraction 2, and Q_2 is the amount of Compound 2 contained in Fraction 1 (Fig. 2). For more convenience, Ti_1 and Ti_2 can be rewritten in terms of $\eta_1 = Q_1/Q_{0_1}$ and

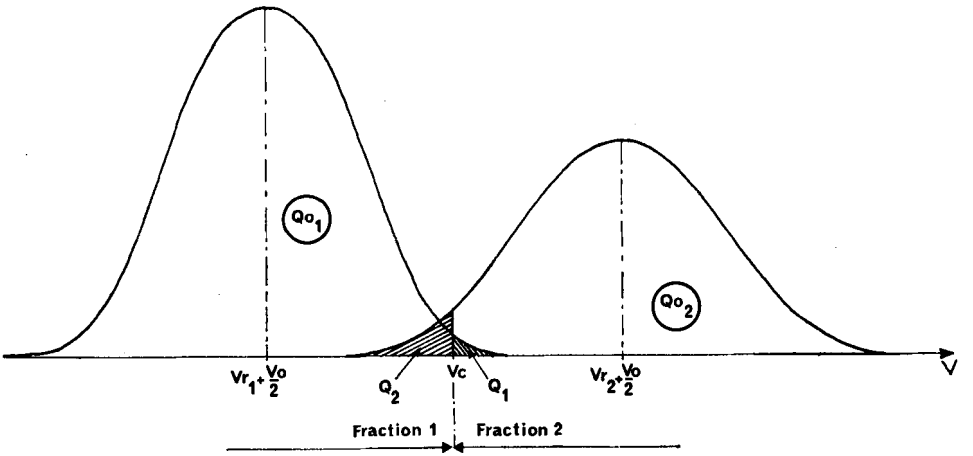

FIG. 2. Definition of impurity ratios Ti_1 and Ti_2 for two partially overlapped peaks separated into two fractions:

$$Ti_1 = \frac{Q_2}{Q_{0_1} - Q_1} \qquad Ti_2 = \frac{Q_1}{Q_{0_2} - Q_2}$$

$\eta_2 = Q_2/Q_{0_2}$:

$$Ti_1 = \frac{C_{0_2}}{C_{0_1}} \frac{\eta_2}{1 - \eta_1}, \qquad Ti_2 = \frac{C_{0_1}}{C_{0_2}} \frac{\eta_1}{1 - \eta_2} \tag{11}$$

η_1 and η_2 are convenient intermediate variables since they are also concerned with the expression of recovery ratio Tr (*10*), defined as the ratio of the recovered quantity Qr to the injected quantity Q_0 of a compound. So we obtain

$$Tr_1 = 1 - \eta_1, \qquad Tr_2 = 1 - \eta_2$$

With Gaussian analytical peaks for both compounds, Ti_1 and Ti_2 can be numerically calculated from knowledge of the second integral of the Gauss function:

$$S(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} \int_{-\infty}^{y} e^{-z^2/2}\, dz\, dy$$

x is a normalized and centered variable. We have tabulated the values of $S(x)$ for x ranging from -4 to 0 (Table 1). η_1 and η_2 are expressed with the function $S(x)$ by

$$\eta_1 = \frac{\sigma_1}{V_0}\left[S\left(\frac{V_{R1} + V_0 - V_c}{\sigma_1}\right) - S\left(\frac{V_{R1} - V_c}{\sigma_1}\right)\right] \tag{12}$$

TABLE 1

$$S(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} \int_{-\infty}^{y} e^{-z^2/2}\, dz\, dy$$

x	$S(x)$	x	$S(x)$
−4	0.000007	−2.0	0.008491
−3.9	0.000011	−1.9	0.011055
−3.8	0.000017	−1.8	0.014276
−3.7	0.000026	−1.7	0.018288
−3.6	0.000039	−1.6	0.023242
−3.5	0.000058	−1.5	0.029307
−3.4	0.000087	−1.4	0.036668
−3.3	0.000127	−1.3	0.045528
−3.2	0.000185	−1.2	0.056103
−3.1	0.000267	−1.1	0.068620
−3	0.000382	−1.0	0.083315
−2.9	0.000542	−0.9	0.100431
−2.8	0.000761	−0.8	0.120207
−2.7	0.001060	−0.7	0.142879
−2.6	0.001464	−0.6	0.168672
−2.5	0.002004	−0.5	0.197796
−2.4	0.002721	−0.4	0.230438
−2.3	0.003662	−0.3	0.266761
−2.2	0.004887	−0.2	0.306894
−2.1	0.006468	−0.1	0.350935
		0	0.398942

$$\eta_2 = \frac{\sigma_2}{V_0}\left[S\left(\frac{V_c - V_{R2}}{\sigma_2}\right) - S\left(\frac{V_c - V_0 - V_{R2}}{\sigma_2}\right)\right] \tag{12}$$

When V_0 is larger than $4\sigma_2$, the second term between the brackets is negligible. Relationships (11) and (12) are very important in practice since they allow the numerical computations of the impurity ratios very easily from the analytical and V_c data only.

As a practical example, the case of V_0 equal to $V_{0_{lin}}$ ($Rs' = 1$) will now be dealt with. In that case, the inflection tangents intersect each other on the base line at the elution volume:

$$V_{R1} + \frac{V_0}{2} + \frac{W_1'}{2} = V_{R2} + \frac{V_0}{2} - \frac{W_2'}{2}$$

It is convenient to take this elution volume for V_c. The values of Ti_1 and Ti_2 have been calculated in terms of $V_{0_{lin}}$ and the σ_2/σ_1 ratio, for an equimolar mixture ($C_{0_1} = C_{0_2}$). The results are given in Fig. 3. This figure shows us that the impurity ratio decreases as V_0 increases, although the

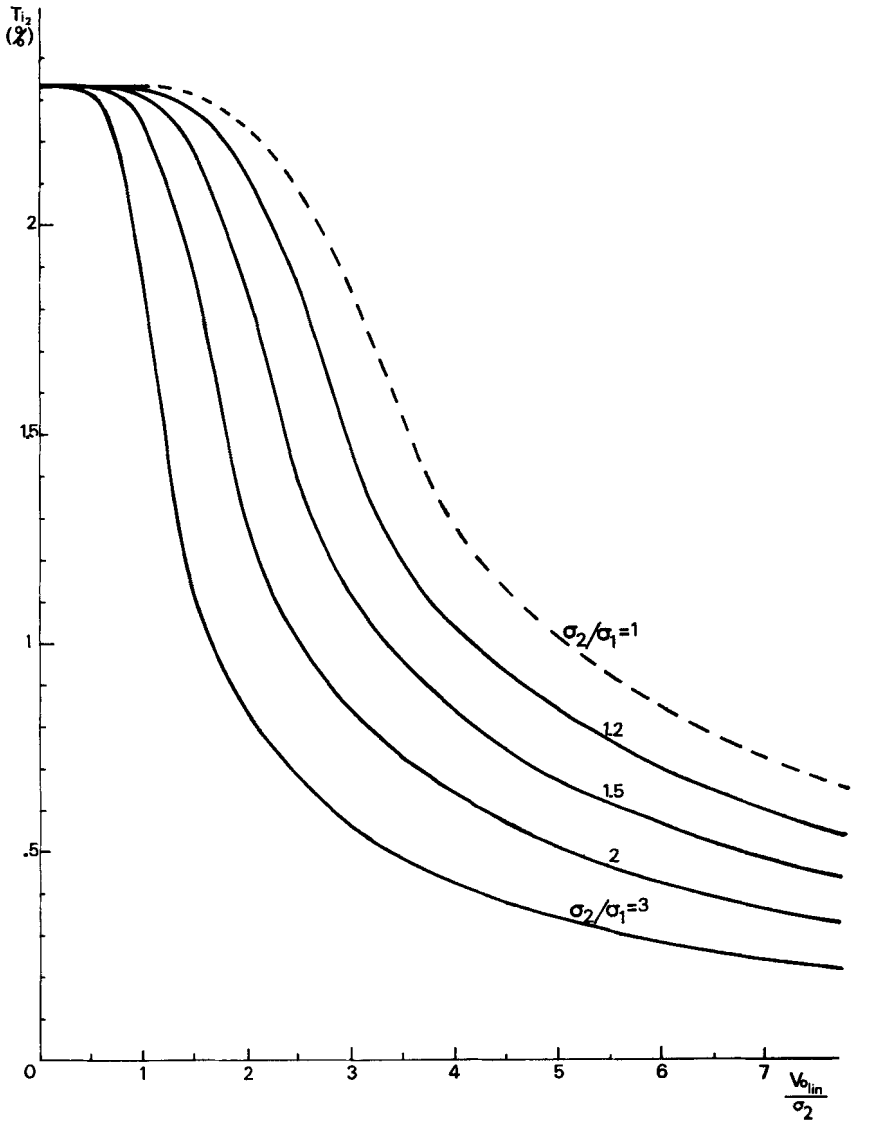

FIG. 3. Variations of the impurity ratios (Ti_1 and Ti_2) with the sample volume $V_{0_{lin}}$ defined by $Rs' = 1$. Binary mixture separated into two fractions, 1 and 2. Gaussian impulse responses (standard deviations: σ_1 and σ_2). Pulse-shaped injections: $C_{0_1} = C_{0_2}$. With the choice of the reduced variable $V_{0_{lin}}/\sigma_2$, the dashed curve also depicts Ti_1 whatever the σ_2/σ_1 ratio.

preparative resolution Rs' remains equal to unity. For the lowest $V_{0_{lin}}$, Ti_1 and Ti_2 go up to the well-known Gaussian impurity ratio (2.3% for $C_{0_1} = C_{0_2}$). For the largest $V_{0_{lin}}$ ($V_{0_{lin}} > 4\sigma_2$ or even $Rs > 1.3$) expressions, Ti_1 and Ti_2 reduce to

$$Ti_1 = \frac{C_{0_2}}{C_{0_1}} \frac{\sigma_2}{V_0} S\left(-\sqrt{\frac{\pi}{2}}\right) \quad \text{and} \quad Ti_2 = \frac{C_{0_1}}{C_{0_2}} \frac{\sigma_1}{V_0} S\left(-\sqrt{\frac{\pi}{2}}\right)$$

with

$$S\left(-\sqrt{\frac{\pi}{2}}\right) = S(-1.25) = 0.05$$

It results from this figure that, for a given resolution, the purity is always better in preparative than in analytical chromatography. This fact makes it feasible to get material of high purity even in the case of poor preparative resolution.

In the Appendix the reader will find an illustrative example of impurity and recovery ratio calculations using the foregoing relationships.

Finally the fact that, in preparative chromatography, the impurity ratio varies with both resolution and sample volume has to be underlined again. In other words, for a given resolution, the impurity ratio is not constant as in analytical chromatography (see the initial horizontal slope of the curves in Fig. 3). As a matter of fact, and in agreement with Conder (*12*) and Guiochon (*13*), it appears that resolution is not the most suitable concept to define the purity constraint in preparative chromatography.

APPENDIX: EXAMPLE OF IMPURITY AND RECOVERY RATIO CALCULATIONS

The numerical chromatographic data for this example are based on the following actual experimental separation conditions.

Solute 1: resorcinol ($C_{0_1} = 10^{-2}\ M$)
Solute 2: phenol ($C_{0_2} = 5 \times 10^{-3}\ M$)
Column: 25 cm × 7.6 mm (3/8 in. o.d.)
Stationary phase: Lichroprep RP 8 25–40 μm (Merck, Darmstadt-RFA)
Mobile phase: methanol-water (65:35 v/v)

An analytical injection gives

$$V_{R1} = 9.1\ \text{mL}, \qquad \sigma_1 = 0.34\ \text{mL}$$
$$V_{R2} = 11.7\ \text{mL}, \qquad \sigma_2 = 0.44\ \text{mL}$$

Analytical resolution:

$$Rs = \frac{V_{R2} - V_{R1}}{2(\sigma_1 + \sigma_2)} = 1.67 \qquad (Rs > 1.3)$$

Sample volume corresponding to $Rs' = 1$:

$$V_{0_{\text{lin}}} = 2(\sigma_1 + \sigma_2)\left(Rs - \sqrt{\frac{\pi}{8}}\right) = 1.62 \text{ m}L$$

Resorcinol base width resulting from a $V_{0_{\text{lin}}}$ injection:

$$W_1' = V_{0_{\text{lin}}} + \sigma_1\sqrt{2\pi} = 2.47 \text{ m}L$$

Cut volume:

$$V_c = V_{R1} + \frac{V_{0_{\text{lin}}}}{2} + \frac{W_1'}{2} = 11.15 \text{ m}L$$

Intermediate ratios η_1 and η_2:

$$\eta_1 = \frac{\sigma_1}{V_0}\left[S\left(\frac{V_{R1} + V_{0_{\text{lin}}} - V_c}{\sigma_1}\right) - S\left(\frac{V_{R1} - V_c}{\sigma_1}\right)\right]$$

$$= \frac{\sigma_1}{V_0}[S(-1.25) - S(-6.03)]$$

$$\eta_2 = \frac{\sigma_2}{V_0}\left[S\left(\frac{V_c - V_{R2}}{\sigma_2}\right) - S\left(\frac{V_c - V_{0_{\text{lin}}} - V_{R2}}{\sigma_2}\right)\right]$$

$$= \frac{\sigma_2}{V_0}[S(-1.25) - S(-4.93)]$$

Table 1 shows that $S(-6.03)$ and $S(-4.93)$ are negligibly small, so

$$\eta_1 = \frac{\sigma_1}{V_0}S(-1.25) = 1.05 \times 10^{-2}$$

$$\eta_2 = \frac{\sigma_2}{V_0}S(-1.25) = 1.36 \times 10^{-2}$$

Impurity ratios:

$$Ti_1 = \frac{C_{0_2}}{C_{0_1}}\frac{\eta_2}{1 - \eta_1} = 0.7\%$$

$$Ti_2 = \frac{C_{0_1}}{C_{0_2}}\frac{\eta_1}{1 - \eta_2} = 2.1\%$$

Recovery ratios:

$$Tr_1 = 1 - \eta_1 = 99.0\%$$
$$Tr_2 = 1 - \eta_2 = 98.6\%$$

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