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ACCURATE CALCULATION OF ADJUSTED RETENTION TIME

II. EVIDENCE AND CONCLUSIONS

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SUMMARY

The validity of a new procedure for calculating the adjusted retention time, t'_R , has been confirmed for various columns and for various n -alkane chain lengths. The method gives unbiased and precise results compared to existing methods, which lead to systematic errors. The influence of the method of calculation of t'_R on the values of the Kováts indices is demonstrated. The ratio, A , of the time differences for neighbouring n -alkanes in a homologous series is constant and matches the data calculated by linear regression. The very high precision of the values calculated for t'_R is demonstrated by the coefficient of variation, which does not exceed 0.87%. From the relation between A and the relative retention it is concluded that A is constant over the whole range of relative retention.

INTRODUCTION

All of the methods of calculating the adjusted retention time are based on an accurate estimation of the dead-time. There are several methods of measurement or of calculation of the dead-time. It has been observed that dead-time calculations are markedly influenced by small changes in retention times¹, with a corresponding effect on the accuracy of the adjusted retention time.

A new procedure for calculating the adjusted retention time, t'_R , was introduced in our previous paper². It is based on an evaluation of time differences for the neighbouring n -alkanes in a homologous series.

$$\Delta_n = t_{RC_n} - t_{RC_{n-1}} \quad (1)$$

$$\Delta_{n-1} = t_{RC_{n-1}} - t_{RC_{n-2}} \quad (2)$$

$$A = \Delta_n / \Delta_{n-1} \quad (3)$$

$$t'_{RC_n} = \sum_1^n \Delta_i \quad (4)$$

The adjusted retention time is related to a substance with Kováts index $I = 0$; there is no need to measure the gross retention time, or to determine the instant of injection, etc.

The present paper compares the new procedure with the existing methods of calculating the adjusted retention time, and demonstrates its general validity and applicability.

EXPERIMENTAL

Experiments were carried out on Packard-Becker 428 and Perkin-Elmer F 22 instruments, both equipped with a flame ionization detector (FID). The following columns were used.

Packed. (1) Glass (1.8 m \times 3 mm I.D.), 10% SE-30 on Supelcoport (80–100 mesh) at 100°; (2) stainless steel (1.8 m \times 2 mm I.D.), 10% OV-17 on Supelcoport (80–100 mesh) at 100°; (3) stainless steel (2.0 m \times 2 mm I.D.), 14% Carbowax 20M on Chromosorb W (80–100 mesh) at 100°.

Micro-packed. Glass (3.0 m \times 0.8 mm I.D.), 1.5% OV-17 and 2.0% OV-210 on Kieselguhr (100–120 mesh) at 85°.

Open tubular. Stainless steel (18.0 m \times 0.25 mm I.D.), OV-101 at 60°.

RESULTS AND DISCUSSION

Comparison of various methods for calculating the adjusted retention time

The values of the adjusted retention time, calculated by means of the measured dead-time and calculated dead-time, are given in Table I. A capillary column was used for this comparison, thus avoiding the effect of the separation parameters on the precision of the time differences. It can be seen that the shortest net retention times are those calculated by means of the methane dead-time, since the net retention time for methane is assumed to be zero. However, from the values in the Table I it can be shown that $t'_{RCH_4} = 1.04^*$ as calculated by the proposed method.

The calculation of the net retention time from a sequence of three *n*-alkanes leads to longer t'_R values, because of the positive curvature of dependence of t_M versus *n* as predicted originally by the authors³. When our method is considered as a standard one, it is easy to show that the adjusted retention times, calculated by means of the

* The adjusted retention time of *n*-octane (*i.e.*, the time corresponding to the period between the indices 0 and 800) is:

$$t'_{800-0} = A_{8-7} \cdot \frac{1 - \left(\frac{1}{A}\right)^8}{1 - \frac{1}{A}} = 315.21 \cdot \frac{1 - \left(\frac{1}{2.2615}\right)^8}{1 - \frac{1}{2.2615}} = 564.25$$

$$t'_{800-100} = A_{8-7} \cdot \frac{1 - \left(\frac{1}{A}\right)^7}{1 - \frac{1}{A}} = 315.21 \cdot \frac{1 - \left(\frac{1}{2.2615}\right)^7}{1 - \frac{1}{2.2615}} = 563.21$$

$$t'_{CH_4} = t'_{800-0} - t'_{800-100} = t'_{100-0} = 1.04$$

TABLE I

RETENTION TIMES (FIRST ROW) AND THEIR ESTIMATED STANDARD DEVIATIONS (SECOND ROW) CALCULATED BY METHODS 1-4* FOR FOUR *n*-ALKANES

The data are mean values from five repeated measurements.

C_n	t_{R_n} (sec)	t'_{R_n}			
		1	2	3	4
5	180.54	46.04	48.30	50.44	48.03
	0.195	0.207	0.283	0.681	0.205
6	242.04	107.56	109.82	111.96	109.66
	0.358	0.358	0.477	0.618	0.205
7	381.94	247.44	249.70	251.84	249.04
	0.699	0.677	0.778	0.850	0.390
8	696.58	562.08	564.34	566.48	564.25
	1.915	1.914	2.060	1.660	1.324

* Methods:

$$(1) t'_{R_n} = t_{R_n} - t_{RCH_4}$$

$$(2) t'_{R_n} = t_{R_n} - t_M; t_{M_{567}} = \frac{t_{RC5} \cdot t_{RC7} - t_{RC6}^2}{t_{RC5} + t_{RC7} - 2t_{RC6}}$$

$$(3) t'_{R_n} = t_{R_n} - t_M; t_{M_{678}} = \frac{t_{RC6} \cdot t_{RC8} - t_{RC7}^2}{t_{RC6} + t_{RC8} - 2t_{RC7}}$$

$$(4) t'_{R_n} = \Delta_n \cdot \frac{A^{n+1} - A}{A^{n+1} - A^n}; \text{ see eqns. 1, 2, 3 and 8}$$

methane hold-up time, are significantly shorter (on a 99.9% probability level), and those calculated by means of $t_{M_{678}}$ (dead-time calculated from a sequence of C_6 , C_7 and C_8 *n*-alkanes) are significantly longer.

Values of the estimated standard deviations of the adjusted retention times are also given in Table I. It can be seen that our method of calculation (last column) gives the more precise results, since only time differences are used. The accuracy of the existing methods is dependent on the exact detection of the instant of injection, and the net retention time can generally be expressed by the equation

$$t'_{R_n} = (t_{R_n} \pm t_x) - t_M \quad (5)$$

where t_x is the time reading error stemming from the determination of the instant of injection.

In contrast with the biased results for the adjusted retention time expressed by eqn. 5, our concept, using the time differences, is free from these errors and gives accurate adjusted retention times.

$$\Delta_n = (t_{R_n} \pm t_x) - (t_{R_{n-1}} \pm t_x) = t_{R_n} - t_{R_{n-1}} \quad (6)$$

The proposed method is the first to allow use of linear regression, since there is no curvature in the dependence of A vs. n (see below). If linear regression is used for the

calculation of the mean values, there is no difference between values of the net retention times calculated from the sum of the time differences

$$t_{Rn} = A_1 + A_2 + \dots + A_{n-1} + A_n \quad (7)$$

and those from the sum of the geometric progression:

$$t_{Rn} = A_n \cdot \frac{A^{n+1} - A}{A^{n+1} - A^n} = A_n \cdot \frac{1 - \left(\frac{1}{A}\right)^n}{1 - \frac{1}{A}} \quad (8)$$

Dependence of the Kováts indices on the method of calculation of the adjusted retention time

The dependence of the Kováts indices on the absolute values of the net retention time is demonstrated in Table II (the data were obtained on a capillary column). The trends in the mean values of the index I can be related to the various adjusted retention times calculated in Table I. The differences in the I values are not statistically significant in spite of the significant differences in the net retention times at the 99.9% probability level. This fact stems from the mathematical operations, and it can be concluded that the Kováts index is insensitive to the method of calculation of the adjusted retention time.

Linearity of ratio A

The present method of calculating the net retention time is based on the principle of a constant ratio, A , of the time differences for neighbouring n -alkanes in a

TABLE II

DEPENDENCE OF KOVÁTS' INDEX ON OV-101 AT 60° ON THE METHOD OF CALCULATION OF THE ADJUSTED RETENTION TIME*

The data are mean values from five repeated measurements.

Compound	I			
	1	2	3	4
Cyclopentane	567.65	567.37	567.12	567.32
3-Methylpentane	584.54	584.38	584.24	584.30
Benzene	654.18	654.04	653.91	654.18
2,2,3-Trimethylpentane	735.92	735.86	735.80	735.83
2,3,4-Trimethylpentane	751.83	751.77	751.71	751.72
2,3-Dimethylhexane	761.49	761.44	761.38	761.38
3-Methylheptane	774.21	774.17	774.13	774.09
2,2,5-Trimethylhexane	785.08	785.05	785.02	785.05

* Methods:

$$(1) t_{Ri} = t_{Ri} - t_{RCH_4}$$

$$(2) t_{Ri} = t_{Ri} - t_{M_{267}}$$

$$(3) t_{Ri} = t_{Ri} - t_{M_{678}}$$

$$(4) t_{Ri} = t_{R_n} + (t_{Ri} - t_{R_n})$$

homologous series. A mixture of C_9 - C_{20} *n*-alkanes was used to check the constancy of this ratio over a broad range. Separate experiments in narrower ranges of *n*-alkanes, C_5 - C_8 , C_7 - C_{12} , C_{10} - C_{15} and C_{12} - C_{20} , were also carried out. The data obtained are given in Table III.

TABLE III

EVALUATION OF THE ADJUSTED RETENTION TIME BASED ON THE RATIO OF TIME DIFFERENCES BETWEEN NEIGHBOURING *n*-ALKANES IN HOMOLOGOUS SERIES

The quantities in columns 3-7 were calculated from eqn. 1, 3, 9, 7 and 8, 9 and 11 respectively.

C_n	Measured t'_{Rn} (mm)	Δ_n (mm)	A	$\bar{\Delta}_n$ (mm)	t'_{Rn} (mm)	t'_{Rn} (mm)	$r = \frac{t'_{Rn} + 1}{t'_{Rn}}$
20	2598				2564.46	2564.46	
		704		703.56			1.3781
19	1894		1.3804		1860.90	1860.91	
		510		510.86			1.3784
18	1384		1.3747		1350.04	1350.05	
		371		370.94			1.3789
17	1013		1.3792		979.10	979.11	
		269		269.34			1.3795
16	744		1.3724		709.76	709.77	
		196		195.57			1.3804
15	548		1.3803		514.19	514.19	
		142		142.00			1.3815
14	406		1.3786		372.19	372.20	
		103		103.11			1.3832
13	303		1.3377		269.08	269.09	
		77		74.87			1.3856
12	226		1.3750		194.21	194.21	
		56		54.36			1.3888
11	170		1.4000		139.85	139.84	
		40		39.47			1.3931
10	130		1.3793		100.38	100.38	
		29		28.66			1.3996
9	101				71.72	71.72	
				20.81			1.4090
8					50.91	50.90	
				15.11			1.4222
7					35.80	35.79	
				10.97			1.4414
6					24.83	24.83	
				7.97			1.4745
5					16.85	16.84	
				5.78			1.5212
4					11.07	11.07	
				4.20			1.6114
3					6.87	6.87	
				3.05			1.7984
2					3.82	3.82	
				2.21			2.3727
1					1.61	1.61	
				1.61			
0							

As pointed out in our previous paper², $\log \Delta$ must be linearly dependent on C_n . The evaluation of the experimental data (micro-packed column at 85°) gave a straight line with a regression coefficient of 1.0000 for C_{14} – C_{20} . (The theoretical value 1.0000 of the regression coefficient can be due to the transformation of the time differences into their logarithms.)

$$\log \bar{\Delta}_n = 0.0673 + 0.1390C_n \quad (9)$$

From eqn. 9, the mean values of the time differences may be calculated, together with the mean value of ratio A :

$$\log \bar{A} = \log \bar{\Delta}_n - \log \bar{\Delta}_{n-1} = 0.1390 \quad (10)$$

$$\bar{A} = 1.3772 \quad (11)$$

The arithmetic mean, \bar{A} , for the same column and the same conditions (Table III) was also calculated from the directly measured A and found to be 1.3775 with an estimated standard deviation of $s = 0.0030$. The two results are in very good agreement. The A values of 1.3377 and 1.4000 were excluded by the outlier tests and therefore were not used in the linear regression calculation.

Relative retention and A

It is known that the relative retention, $r = t'_{R_{n+1}}/t'_{R_n}$, is constant at high values of n , but for smaller values of n this is no longer true. The value of r increases with decreasing retention time⁵. This effect is generally caused by the dependence of the activity coefficients on the number of carbon atoms in a molecule. When the dependence of the relative retention on the number of carbon atoms in a molecule is analyzed by the non-linear regression method we find a general equation of the form

$$r = K_0 + \frac{K_1}{n} + \frac{K_2}{n^2} \quad (12)$$

where K_0 , K_1 and K_2 are constants. Now r can be expressed by the following equations:

$$r_1 = \frac{t'_{R_2}}{t'_{R_1}} = \frac{\Delta_2 + \Delta_1}{\Delta_1} = \frac{\Delta_1(A+1)}{\Delta_1} = A + 1 \quad (13)$$

$$r_2 = \frac{t'_{R_3}}{t'_{R_2}} = \frac{\Delta_3 + \Delta_2 + \Delta_1}{\Delta_2 + \Delta_1} = \frac{A^2\Delta_1 + A\Delta_1 + \Delta_1}{A\Delta_1 + \Delta_1} = A + 1 - \frac{A}{A+1} \quad (14)$$

Thus it can be seen that the value of the relative retention is $A + 1$ for the first term and approaches A with increasing alkane chain length. Table III gives the values of r calculated for 20 alkanes, which are in excellent agreement with the above analysis. The constants in eqn. 12 for the relative retentions in Table III were found by poly-

nomial fitting: $K_0 = 1.3227$, $K_1 = 0.7500$ and $K_2 = 0.3048$. Therefore, according to eqn. 12, the relative retention for the first term is:

$$r_1 = 1.3227 + \frac{0.7500}{1} + \frac{0.3048}{1} = 2.3775 \quad (15)$$

$$r_1 = A + 1 = 1.3772 + 1 = 2.3772 \quad (16)$$

Therefore it can be concluded that ratio A is independent of the alkane chain length and is only randomly distributed.

Statistical evaluation

The constancy of ratio A was verified by prolonged measurements. First we attempted to distinguish between the values measured over the whole homologous series and those corresponding to the ratio between the same n -alkanes in repeated measurements. In this way we could determine the reproducibility of one experiment, *i.e.*, the constancy of A , and the reproducibility for the given system under the given conditions (stationary phase, flow, temperature). Some data are given in Table IV.

TABLE IV

ESTIMATED STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR RATIO A IN HOMOLOGOUS SERIES (1) AND IN REPEATED MEASUREMENTS (2)

	Conditions	N	\bar{A}	s	v (%)
1	n -C ₅ -C ₂₀ on OV-17 + OV-210	10	1.3775	0.0030	0.22
1	n -C ₁₀ -C ₁₅ on SE 30	4	1.6957	0.0124	0.73
2	n -C ₁₁ -C ₁₃ on Carbowax 20 M	20	1.7558	0.0154	0.87
2	n -C ₁₁ -C ₁₃ on OV-17	14	1.8900	0.0162	0.85
2	n -C ₅ -C ₈ on OV-101	10	2.2615	0.0141	0.62

The precision of ratio A measured over the series is better than that for the repeated experiments, but in both cases it is very good and the coefficient of variation does not exceed 1%. The somewhat poorer precision for measurements on the SE-30 column is caused by the fact that the time differences were read to only two significant figures, *i.e.*, with a precision poorer than 1%. When the precision of the readings is improved, the overall precision also rapidly improves (see the OV-17 + OV-210 column). We conclude that the precision of the first step (time reading) is the predominant source of random deviations.

Another question is how many values of the ratio A should be used for calculation of the mean. Using a sequence of three n -alkanes, one value of ratio A can be calculated and then the net retention time. By this procedure the accuracy of the adjusted retention time is improved, but the precision remains similar to that of existing methods. (The data used for the calculation were in both cases single data

without the associated relation to the confidence interval). The precision of values of t'_R calculated by the previous methods can only be improved by repeating the experiment. This is time consuming and expensive, and the improvement in precision is small owing to long-term changes in temperature, pressure, etc. The proposed method offers a large improvement in precision within one experiment.

From the values of A in Table III (column 4), it can be seen that spreading of the value of A and of the calculated value of t'_R occurs, if only three n -alkanes are used. (The given distribution is related to the uncertainty in the net retention time as determined by the previous methods.) From the given data, the maximum absolute error for a single value of A is $+0.0029$ (0.21 %) or -0.0051 (-0.37 %) with respect to the mean value of 1.3775. By introducing another value of the ratio A (the data from Table III, column 4, are used in series), the precision of the calculation rapidly increases (see Table V). However, the introduction of further values does not lead to a significant improvement in the accuracy (match of \bar{A}' with \bar{A}), but the precision of the mean value \bar{A}' increases as demonstrated by the corresponding 95 % confidence limits (see Fig. 1). It can be seen that the confidence limits decrease very sharply, but after $N = 5$ the changes are very small. The same result is obtained when Shannon's information content⁴ is used:

$$I(S) = \log_2 \frac{(x_1 - x_0) \sqrt{N}}{2st_{0.039(N_A-1)}} \quad (17)$$

where s is the estimated standard deviation and $t_{0.039(N_A-1)}$ is a critical value of the Student distribution for $\alpha = 0.039$ and for $N_A - 1$ degrees of freedom.

TABLE V

DEPENDENCE OF THE MEAN VALUE OF RATIO A ON THE NUMBER OF VALUES OF A IN HOMOLOGOUS SERIES TAKEN INTO ACCOUNT IN ITS CALCULATION

$$s = (A_{\max} - A_{\min})/k_N; \nu = 100 \cdot s/\bar{A}_N.$$

N	\bar{A}_N	s	ν (%)	Absolute error*	Relative error* (%)
2	1.3776	0.0040	0.29	+0.0001	+0.007
3	1.3781	0.0030	0.22	+0.0006	+0.044
4	1.3767	0.0038	0.27	-0.0008	-0.058
5	1.3774	0.0036	0.26	-0.0001	-0.007
6	1.3776	0.0033	0.24	+0.0001	+0.007
7	1.3772	0.0032	0.23	-0.0003	-0.022
8	1.3772	0.0032	0.23	-0.0003	-0.022
9	1.3775	0.0030	0.22	0.0000	0.000
10	1.3775	0.0030	0.22	0.0000	0.000

* Relative to $1.3775 = \bar{A}$.

From Fig. 2 it can be seen that the information content $I(S)$ for more than five values of A increases only slowly. Thus it is worthless to increase the number of values of ratio A considered, since the number required to significantly improve the precision (i.e., to obtain a coefficient of variation of 0.10 %) will be 193 at the 95 % significance level.

If the proposed method is compared with the existing methods, the advantage of the use of linearization and in this way the calculation of the mean must be pointed out, in contrast to the single data obtained by previous methods, which are subject to gross errors.

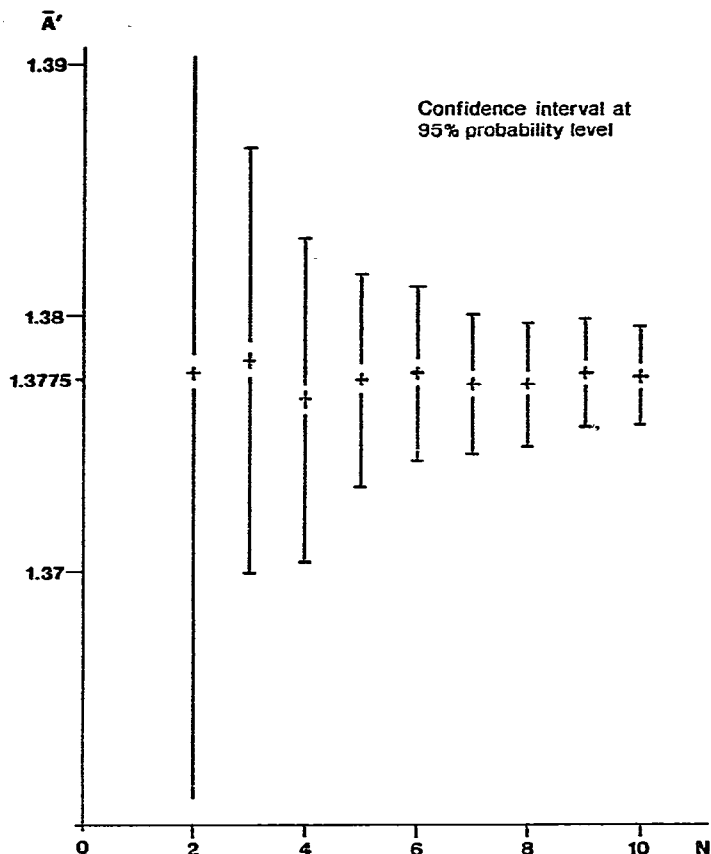


Fig. 1. Dependence of the mean value and 95% confidence limits for A on the number of values of A employed in the calculation of the mean.

CONCLUSIONS

It is concluded that the calculation of the adjusted retention time by means of the ratio of the time differences for neighbouring n -alkanes in homologous series gives unbiased and precise results compared with the existing methods of calculation which lead to systematic errors. The precision of the calculation is determined by the precision of reading single (measured) time differences, which in the proposed method is improved by the linear regression method. The linear regression method gives the mean values of the time differences Δ as well as the mean of ratio A . For the recalculation to the adjusted retention time the sum of geometric series (progression) can be easily applied.

The dependence of the Kováts indices on the method for the calculation of the net retention time was also studied. Even with significantly biased net retention times, no statistically significant differences in the Kováts index values were found.

The computed ratio, A , of the time differences for neighbouring members of a homologous series was found to be constant and independent of the n -alkane chain length. A good reproducibility of A was demonstrated for homologous series as well

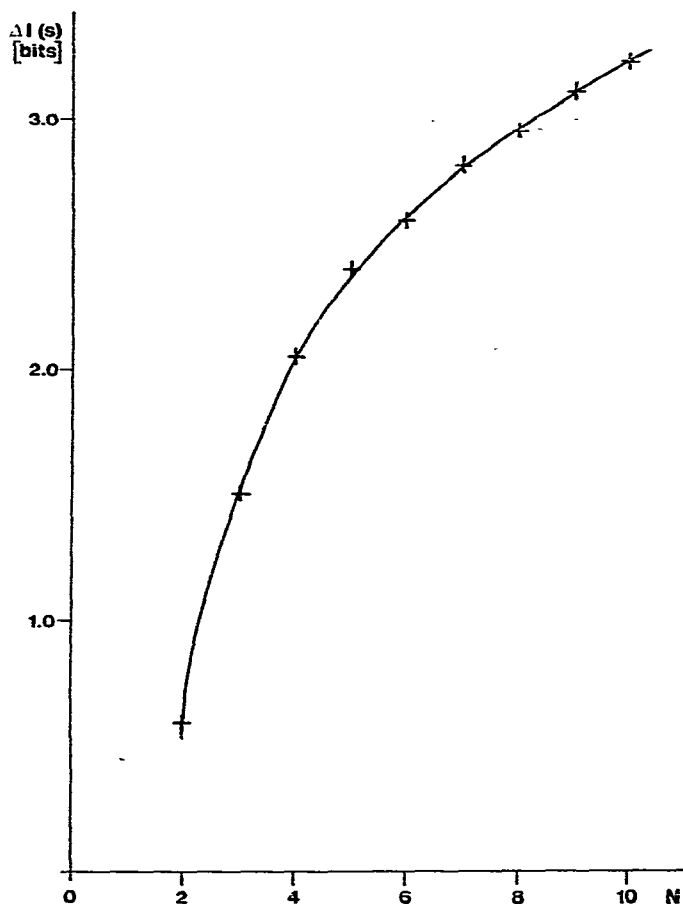


Fig. 2. Dependence of the information content $\Delta I(S)$ of the experimental arrangement on the number of values of A taken into account.

as for repeated analyses. The poorest coefficient of variation was 0.87%. Statistical evaluation showed that there is no sense in increasing the number of ratios calculated over five.

The relationship between A and the relative retention r was demonstrated and the limiting values were found. With constancy of A , the relative retention equals $A + 1$ for small chain lengths and approaches A for long chains. Statistically significant differences in the values of ratio A for different stationary phases were found. Owing to the independence of A on n -alkane chain length, A can be used for classification of stationary phases. This problem is further studied.

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