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## SIMULTANEOUS PARAMETER COMPENSATION IN THE REPLICATION OF PROGRAMMED TEMPERATURE GAS CHROMATOGRAPHIC RETENTION MEASUREMENTS

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### SUMMARY

Tables of relative retention times, retention indices or emergence temperatures obtained under one set of chromatographic conditions can be used for the tentative identification of unknowns determined under an entirely different set of conditions. This can be accomplished for a particular liquid phase if the emergence temperature of a reference compound is maintained while one or more chromatographic parameters are changed to compensate for the changes in other parameters. The advantage of using proximal standards for the determination of both relative retention times and emergence temperatures is demonstrated in this report.

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### INTRODUCTION

The initial, tentative identification of unknowns detected by gas chromatography is often obtained by searching published retention data for compounds which have the same retention at equivalent chromatographic conditions. When the chromatographic retention of the unknown is determined, the column temperature, as well as the nature of the stationary phase, must be the same as that used to obtain the published data otherwise precise duplication of the retention data may not occur.

It is especially important that the column temperature be the same if the molecular shape of the unknown differs from that of the reference compound. If their molecular shapes differ, the retention time of one relative to the other will likely be temperature dependent<sup>1</sup>. For example, Bilen and Mikkelsen<sup>2</sup> observed that the retention of acenaphthylene relative to that of normal pentadecane is temperature dependent during isothermal gas chromatography (IGC) even when the two compounds emerge close to one another. Acenaphthylene emerged prior to pentadecane at a column temperature of 165°C and emerged after the normal hydrocarbon when the column was operated above 175°C. In addition, Harris and Habgood<sup>3</sup> demonstrated a similar temperature dependency, during programmed temperature gas chromatography (PTGC) between the Kováts index of a cycloalkane and its emergence temperature. Again, as its emergence temperature was increased, the ring compound emerged at a later time relative to the adjacent straight chain hydrocarbon.

In the practical application of PTGC to the analyses of samples for pesticide and industrial chemical residues, a similar temperature dependency will frequently occur because of the wide range of molecular shapes encountered. Thus if a precise duplication of tabulated retention data is to be obtained so a tentative identification of the residue can be made, it is essential that the emergence temperature of the unknown closely duplicate that which it attained when the data was generated.

In PTGC the most common technique used to ensure a duplication of retention data is to duplicate all the column parameters that were used when the retention data were produced. This includes a duplication of the type of stationary phase, column dimensions, amount of stationary phase, carrier flow-rate and rate of temperature increase. Since the conditions are strictly adhered to in order to obtain acceptable results, flexibility in the choice of conditions for the analysis of an unknown is only possible if more than one table of retention data is available in which each is based on different chromatographic parameters.

Knöppel *et al.*<sup>4</sup> circumvented the temperature dependency problem another way. They showed by mathematical analysis that reproducible retention indices could be obtained if columns with the same type of stationary phase and phase ratio (the ratio of the column gas phase volume to liquid phase volume) were used and if the starting temperature and elution (emergence) temperatures remain fixed. They further deduced that the temperature programming rate, the column inlet to outlet pressure ratio and the column geometry need not be standardized to obtain reproducible retention indices as long as the emergence temperatures are maintained by appropriately adjusting some parameters to compensate for changes in others.

Saxton<sup>5</sup> demonstrated that if columns are normalized by this technique of simultaneous parameter compensation so that the emergence temperature of a designated reference compound is reproduced, the emergence temperatures of all other compounds are also reproduced even though all the parameters, except the nature of the stationary phase, are varied. It is further noted that emergence temperatures, as well as retention indices, could be determined using one set of PTGC conditions, and the results tabulated and then used for the identification of unknowns determined under a different but equivalent set of conditions.

If, as indicated, reproducible retention indices and emergence temperatures can be obtained when simultaneous parameter compensation is used to normalize column conditions, then reproducible relative retention times certainly should be obtainable by the same technique. This hypothesis was tested on a variety of compounds and on columns with widely different operating parameters. The precision of the relative retention times is presented here, along with comparative information for emergence temperatures which were calculated from the same data.

Colby *et al.*<sup>6</sup> have shown that the precision of retention measurements improves as the sample and reference compounds emerge ever closer together during PTGC. Certainly, the close proximity between the sample and reference compounds is one reason retention indices, such as the Kováts indices, can be so precisely reproduced. However, proximal reference compounds can also be used to a similar advantage in the determination of emergence temperatures and relative retention times as will be shown in this report.

## MATERIALS AND METHODS

The method used for this study was identical to that used previously<sup>5</sup> with the following addition: The 36-m capillary column was used in a Hewlett-Packard 5880 gas chromatograph which was operated to maintain a constant, linear flow-rate. The operating conditions are listed in Table I for chromatographic column 8b.

As before, emergence temperatures were calculated by the equation

$$T_{e1} = T_{e2} + r' (t_{R2} - t_{R1}) \quad (1)$$

where  $T_{e1}$  is the emergence temperature of the analyte of interest,  $T_{e2}$  is the tabulated emergence temperature of the reference compound,  $r'$  is the standardized temperature program rate<sup>5</sup> and  $t_{R2} - t_{R1}$  is the difference in retention time between the analyte of interest and the reference compound. The subscripts have been changed from those used in the previous publication so they will coincide with the IUPAC symbols<sup>7</sup> of the next two equations.

Relative retention times were calculated by the equation

$$r_{1/2} = t'_{R1}/t'_{R2} \quad (2)$$

where  $t'_{R1}$  is the adjusted retention time of the analyte of interest and  $t'_{R2}$  is the adjusted retention of the reference compound, chlorpyrifos.

## RESULTS AND DISCUSSION

Table I lists compounds selected to rigorously test the precision of retention measurements. The compounds represent a wide range of molecular weights (142 to 505) as well as differences in polarity and molecular shape. As noted in the Introduction one of the compounds, acenaphthylene, is highly temperature dependent. A variety of pesticides is included to demonstrate the practical application of the technique.

The columns listed in Table I were selected to test a range of column dimensions, amounts of stationary phase, carrier gasses, flow-rates and temperature program rates. Three different gas chromatographs, each with a different type of detector, were used. Columns 1 through 7 were packed columns, and column 8 was a wall coated open tubular (capillary) column.

As shown in Table II, the coefficient of variation (C.V.) for chromatographic columns 1 through 7 demonstrates good precision for relative retention times. However, better precision was obtained for the emergence temperatures even though both sets of data were determined from the same chromatograms. One reason for this is that emergence temperatures are independent of the initial column temperature if the compound is cold trapped, whereas relative retention times are affected by that parameter whether or not the compound is cold trapped. Thus at least one more variable exists in the determination of relative retention times.

When results from capillary column 8a are included in the calculations, the C.V. of the relative retention times becomes considerably worse compared with the results of the packed columns alone (1 through 7) even though the precision of the

TABLE I  
EMERGENCE TEMPERATURES,  $T_e$ , AND RELATIVE RETENTION TIMES,  $r_{1/2}$ , OF SEVERAL TYPES OF COMPOUNDS ON A VARIETY OF CHROMATOGRAPHIC COLUMN CONDITIONS

Chromatographic column No.									
	1	2	3	4	5	6	7	8a	8b
Inside diameter (mm)	4	4	4	4	4	4	2		0.25
Length (cm)	180	180	120	44	44	180	180	3600	
Amount of liquid phase (%)	5	5	5	10	3	5	5		0.2 $\mu$ m
Carrier gas	He	N <sub>2</sub>	N <sub>2</sub>	N <sub>2</sub>	N <sub>2</sub>	He	He	H <sub>2</sub>	
Flow-rate (ml/min)	49	56	51	34	25	64	35/33	41/31	41
$r'$	2.80	3.15	4.85	4.19	10.06	4.07	4.70	3.12	3.46
$t_R$ of <i>n</i> -C <sub>20</sub> (min)	53	48	31	37	15	39	—	46	43
<i>n</i> -Decane									
$T_e$	84.2	83.7	84.7	83.2	86.0	78.5	—	85.1	85.4
$r_{1/2}$	0.217	0.220	0.229	0.236	0.228	0.236	—	0.167	0.200
Acenaphthylene									
$T_e$	136.9	136.7	137.8	137.0	138.1	135.2	—	136.3	137.8
$r_{1/2}$	0.573	0.575	0.584	0.588	0.581	0.592	—	0.538	0.566
<i>n</i> -Hexadecane									
$T_e$	159.6	159.5	160.1	159.7	160.5	159.1	—	159.6	160.1
$r_{1/2}$	0.727	0.728	0.733	0.737	0.732	0.743	—	0.707	0.721
<i>n</i> -Eicosane									
$T_e$	200.0	200.0	200.0	200.0	200.0	200.0	—	200.0	200.0
$r_{1/2}$	1.000	1.000	1.000	1.000	1.000	1.000	—	1.000	1.000
1-Octadecanol									
$T_e$	207.6	207.2	205.8	205.9	206.7	207.9	—	205.9	206.5
$r_{1/2}$	1.051	1.048	1.039	1.039	1.046	1.050	—	1.043	1.045
<i>n</i> -Docosane									
$T_e$	217.6	217.6	217.3	217.6	217.2	217.4	—	217.6	217.8
$r_{1/2}$	1.119	1.118	1.115	1.115	1.117	1.110	—	1.128	1.124
<i>n</i> -Tetracosane									
$T_e$	233.8	233.8	233.2	233.7	233.9	233.3	—	233.8	234.2
$r_{1/2}$	1.228	1.227	1.222	1.220	1.223	1.210	—	1.245	1.239



emergence temperatures is not adversely affected (data column 2, Table II). The disparity in relative retention times between the capillary and packed columns can be attributed to the change in flow-rate that occurred during the capillary column determination. The capillary column 8a was operated by the common practice of using a constant head pressure and, as to be expected, the carrier gas flow through the column varied inversely with the temperature. As a result of this, early compounds emerged several degrees lower and late compounds emerged several degrees higher than on the normalized packed columns where a constant flow was maintained. This effect was compensated for in the calculation of emergence temperatures by use of the standardized temperature program rate,  $r'$  (ref. 5). When the capillary column was operated to maintain a constant flow-rate rather than a constant head pressure, the relative retention time results more closely agreed with those from the packed columns (chromatographic column 8b of Table I and the C.V. for data column 3, Table II). Thus if the retention data are to be used interchangeably between packed and capillary columns, and maximum precision is to be obtained, both columns must be operated in the same manner—either at constant flow or at constant head pressure.

Proximal reference compounds can be used in the determinations of both the emergence temperatures and the relative retention times of unknowns to enhance the precision with which those retention measurements match tabulated data. When the emergence temperature of an unknown is determined, any tabulated compound that emerges close to it can be used as the reference compound and the results calculated by eqn. 1. However, when relative retention times are determined, eqn. 2 can be used only if the reference compound is the same as that used to obtain the published data. If the proximal reference compound is different, then relative retention times can be calculated by the equation

$$r_{1/2} = r_{3/2} t'_{R1} / t'_{R3} \quad (3)$$

where the subscript 1 refers to the unknown, 2 refers to the reference compound used to obtain the tabulated data and 3 refers to the proximal reference compound. Thus  $r_{1/2}$  is the retention time of the unknown relative to the retention time of the reference compound which was used to obtain the tabulated data, e.g., chlorpyrifos,  $r_{3/2}$  is the tabulated relative retention time of the proximal reference compound,  $t'_{R1}$  is the adjusted retention time of the unknown and  $t'_{R3}$  is the adjusted retention time of the proximal reference compound.

The precision for the emergence temperatures and relative retention times of endosulfan II and pp'-DDT improved considerably when 11-dichloro-2,2-bis(*p*-chlorophenyl)ethane (pp'-TDE) was used as a proximal reference compound (Table III) in comparison with use of the more distantly emerging reference compound, chlorpyrifos. Likewise, the results for *trans*-permethrin and testosterone cypionate improved when they were recalculated from the proximal standard, delta-permethrin (Table III). For this demonstration, the retention values obtained from chromatographic column 2 were arbitrarily designated as the published data. The other chromatographic column results were treated much like retention data for unknowns, and proximal reference compounds were used to bring them into closer agreement with the results from chromatographic column 2.

TABLE II

THE COEFFICIENTS OF VARIATION FOR THE RETENTION MEASUREMENTS PRESENTED IN TABLE I

	<i>Chromatographic columns</i>		
	<i>1/7</i>	<i>1/8a</i>	<i>1/7 + 8b</i>
<i>n</i> -Decane			
$T_e$	3.092	2.191	2.956
$r_{1/2}$	3.477	10.978	5.682
Acenaphthylene			
$T_e$	0.743	0.702	0.717
$r_{1/2}$	1.264	3.125	1.566
<i>n</i> -Hexadecane			
$T_e$	0.306	0.282	0.291
$r_{1/2}$	0.812	1.554	0.979
1-Octadecanol			
$T_e$	0.422	0.423	0.391
$r_{1/2}$	0.509	0.473	0.465
<i>n</i> -Docosane			
$T_e$	0.081	0.078	0.096
$r_{1/2}$	0.287	0.492	0.385
<i>n</i> -Tetracosane			
$T_e$	0.160	0.156	0.190
$r_{1/2}$	0.530	0.866	0.721
<i>n</i> -Hexacosane			
$T_e$	0.208	0.201	0.228
$r_{1/2}$	0.700	1.141	0.919
Testosterone cypionate			
$T_e$	0.403	0.614	0.478
$r_{1/2}$	1.356	2.414	1.729
Eptam			
$T_e$	1.407	1.537	1.289
$r_{1/2}$	1.530	4.954	2.720
Lindane			
$T_e$	0.172	0.156	0.154
$r_{1/2}$	0.629	0.862	0.619
Diazinon			
$T_e$	0.225	0.209	0.202
$r_{1/2}$	0.269	0.627	0.403
Endosulfan II			
$T_e$	0.164	0.169	0.180
$r_{1/2}$	0.359	0.571	0.435
pp'-TDE			
$T_e$	0.144	0.151	0.201
$r_{1/2}$	0.408	0.640	0.531
pp'-DDT			
$T_e$	0.148	0.151	0.184
$r_{1/2}$	0.513	0.791	0.615
pp'-Methoxychlor			
$T_e$	0.185	0.178	0.214
$r_{1/2}$	0.675	0.952	0.753
<i>trans</i> -Permethrin			
$T_e$	0.265	0.249	0.276
$r_{1/2}$	0.926	1.254	1.003
$\delta$ -Permethrin			
$T_e$	0.375	0.345	0.389
$r_{1/2}$	1.271	1.632	1.350

TABLE III

EMERGENCE TEMPERATURES,  $T_e$ , AND RELATIVE RETENTION TIMES,  $r_{1/2}$ , OF ENDO-SULFAN II AND pp'-DDT WHEN pp'-TDE WAS USED AS THE PROXIMAL STANDARD AND OF pp'-METHOXYCHLOR AND delta-PERMETHRIN WHEN *trans*-PERMETHRIN WAS USED AS THE PROXIMAL STANDARD

Chromatographic column 2 was arbitrarily selected as the "published data" to which the retention values were brought into closer agreement.

	Chromatographic column No.						
	2	3	4	5	7	8a	8b
Endosulfan II							
$T_e$	213.7	213.5	213.7	213.7	213.4	213.6	213.3
$r_{1/2}$	1.140	1.138	1.140	1.139	1.137	1.137	1.136
pp'-DDT							
$T_e$	224.1	224.0	224.0	223.8	224.0	224.0	223.9
$r_{1/2}$	1.213	1.212	1.210	1.211	1.214	1.215	1.212
pp'-Methoxychlor							
$T_e$	233.9	234.1	233.9	234.3	233.8	234.0	233.9
$r_{1/2}$	1.282	1.284	1.285	1.284	1.277	1.276	1.279
delta-Permethrin							
$T_e$	273.1	272.7	272.7	272.4	273.6	273.1	273.4
$r_{1/2}$	1.557	1.555	1.552	1.555	1.566	1.566	1.563

Many detectors which are used to screen for pesticide and industrial chemical residues respond only to analytes containing one or two of the following elements: phosphorus, sulfur, nitrogen or the halogens. Since any tabulated compound can be used as a reference for the determination of emergence temperatures and relative retention times, appropriate reference compounds can be selected for each particular element specific detector.

TABLE IV

THE COEFFICIENTS OF VARIATION FOR THE RETENTION MEASUREMENTS PRESENTED IN TABLE III

	Chromatographic columns		
	1/7	1/8a	1/7 + 8b
Endosulfan II			
$T_e$	0.066	0.059	0.082
$r_{1/2}$	0.114	0.121	0.143
pp'-DDT			
$T_e$	0.049	0.044	0.046
$r_{1/2}$	0.130	0.154	0.117
pp'-Methoxychlor			
$T_e$	0.085	0.076	0.078
$r_{1/2}$	0.250	0.303	0.249
delta-Permethrin			
$T_e$	0.170	0.155	0.169
$r_{1/2}$	0.120	0.387	0.344



Similarly, when Kováts indices are used, any pair of tabulated compounds that bracket the unknown can be used as reference compounds even though a series of normal hydrocarbons is used as the frame of reference for the tabulated data<sup>8,9</sup>. Therefore, appropriate reference compounds can be selected so Kováts indices can also be determined for unknowns detected with element specific detectors.

It is apparent that all three of the methods, emergence temperatures, relative retention times and Kováts indices, are versatile and can be used to precisely match the retention of an unknown with tabulated data for identification purposes. Certainly, another popular method, that of matching absolute retention times, can be added to the list. Each has certain favorable attributes, many of which are compared in Table V.

The comparisons made in Table V indicate that emergence temperatures and Kováts indices have many positive characteristics in common and are superior to relative retention times and absolute retention times as a tool for determining the identity of unknowns detected during PTGC.

Tables of both the emergence temperatures and Kováts indices list compounds according to fundamental physical values, *i.e.*, their emergence temperatures and their emergence relative to the normal hydrocarbons, respectively. However, in PTGC emergence temperatures are the more significant of the two. Harris and Habgood<sup>3</sup> have described the retention temperature (the emergence temperature) as the most valuable retention parameter. They further state that it is always measured for reliable identification in PTGC. This has been substantiated in the successful application of simultaneous parameter compensation, whether to facilitate the use of Kováts indices, relative retention times or emergence temperatures as a measure of compound retention. Since emergence temperatures can be precisely and simply determined, and are important, fundamental, physical measurements, their use for

TABLE V

## A COMPARISON OF METHODS FOR MEASURING RETENTION DURING PTGC

$T_e$  = Emergence temperature; KI = Kováts index;  $r_{1/2}$  = relative retention time and  $t_R$  = absolute retention time.

	$T_e$	KI	$r_{1/2}$	$t_R$
Can be used with any column of the same liquid phase and normalized by simultaneous parameter compensation	×	×	×	
Can be used with any element selective detector	×	×	×	×
Reference compound may be proximal to the unknown	×	×	×	
Retention time of an unretained compound need not be determined	×	×		×
Unaffected by the duration of the initial temperature for compounds that are cold trapped	×	×		
Independent of the starting temperature for compounds that are cold trapped	×	×		

measuring compound retention during PTGC determinations certainly merits consideration.

## CONCLUSIONS

This report demonstrates that simultaneous parameter compensation can be used to obtain reproducible relative retention times as well as emergence temperatures, thereby allowing a wide variety of column dimensions and operating parameters to be used. Since it has been reported that this same technique can be used for the determination of Kováts indices<sup>4</sup> it is apparent that any one of these three methods can be used to precisely characterize compounds determined by PTGC while allowing flexibility in the choice of column parameters so that they can be selected to best suit each situation. Thus a single table of emergence temperatures, relative retention times or Kováts indices can be used in the identification of compounds detected using a short column and the conditions appropriate for rapid screening analysis, and can also be used in the identification of compounds detected on a longer column set up to provide better resolution but with a compensatory increase in the analysis time.

Some of the features of various methods of measuring chromatographic retention were compared with one another in this report to provide a basis for the selection of methods for the identification of unknowns. Kováts indices and emergence temperatures fared the best. Of the two, the latter are simpler and more basic to PTGC processes.

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