We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Features and New Examples of Gas Chromatographic Separation of Thermally Unstable Analytes

Igor G. Zenkevich

Abstract

The processes of thermal decomposition of analytes in gas chromatographic (GC) columns are classified and two new examples of them are considered in details. First of them is monomolecular decomposition of monoalkyl esters of benzene-1, 2-dicarboxylic (phthalic) acid (monoalkyl phthalates). This process has the analogy in chemical reactions in solutions and it may be responsible for the toxicity of phthalates. The second example is decomposition of non-substituted hydrazones of both aliphatic and aromatic carbonyl compounds. The analytes of the second sub-group present the first example of bimolecular (second order) decomposition in a GC column: two molecules of hydrazones form stable azines and hydrazine. Besides that this process presents the particular interest, because it is accompanied by secondary chemical reactions not in an injector, but within GC column, when a by-product of decomposition is involved into secondary interaction with other constituents of the samples. It was confirmed, that visual images of all these decomposition processes on the chromatograms are rather identical and coincide with the manifestations of interconversion of isomers or tautomers. The most often expressed features of chromatographic profiles in such cases are the presence of peaks of an initial analyte and a product of its decomposition or isomerization, connected with more or less expressed diffused "plateau" or "train" between them. The decomposition processes during sample preparation prior to chromatographic separation or in the heated injector of GC instrument are not accompanied by such features. Despite of the rather "exotic" character of the examples considered, the knowledge of them seems to be useful for better revealing the analogous situations in chromatographic practice. Thermal instability of analytes is the principal restriction of GC separation of reactive compounds and we cannot eliminate it for objective reasons. However, in some cases we can evaluate the temperature limits of chromatographic columns, which should not be exceeded during GC separation of instable compounds. The simplest (low boiling) homologs of thermally unstable compounds are often characterized by "normal" boiling point at atmospheric pressure (T_b, °C) without decomposition, that means the possibility of their GC analysis unambiguously. Therefore, we can select such T_b values as GC and/or GC–MS temperature limit (T_{lim}) for other members of series of thermally unstable homologs. If GC separation is carried out not in isothermal, but in temperature programming conditions, so-called retention temperature (T_R) of unstable analytes should not exceed the evaluated T_{lim} value.

Keywords: gas chromatography, GC-MS, thermally unstable analytes, new examples of thermal instability, monoalkyl phthalates, non-substituted hydrazones, secondary reactions in GC column, stability of analytes criterion

1. General comments (introduction)

Possible instability of analytes prior or during their separation seems to be the key restriction of chromatographic methods, because it distorts the analytical results and can make them even inacceptable at all due to their irreproducibility and/or incorrectness. The influence of instability due to the different reasons can manifest itself at different stages of analytical procedures, hence it may be considered as a way of classifying them.

The instability of components in samples prepared for analysis (**reason I**). Besides the immanent chemical instability of analytes, it can be possible due to their oxidation by air oxygen or hydrolysis by impurities of water in air or in a solvent. These reasons can be avoided by special sample protection that was demonstrated for chemically high reactive fluoro- and chlorosilanes (hydrolysis), boranes, germanes (oxidation), etc. [1]. Gas Chromatographic Retention Indices (**GC RI**) of such labile compounds determined even at the late 1970s [1] remained unique up to present. The instability during GC separation can be caused by *thermal degradation of unstable analytes in a heated injector* (**reason II**), as well. The similar **reason III** of instability seems to be the *mutual interaction of most reactive components of the samples with each other at the temperatures of GC injection*, if even their mixtures are stable in the samples prepared at the ambient conditions. However, the decomposition processes in the injector can be, if not eliminated, then controlled and sometimes minimized by varying its temperature, as well the application of so-called on-column injection. On the other side, *the decomposition processes within chromatographic columns* (**reason IV**) are usually much more difficult to manage and they represent the most difficulties in chromatographic practice. Often enough, in the result of such processes the analytical parameters (e.g., RIs) of decomposition products are attributed to the initial unstable analytes that are the typical examples of misidentification.

In high performance liquid chromatography (HPLC) the main reason of instability is the interaction of analytes with components of an eluent (most often hydrolysis).

Sometimes the revealing of analytes' instability seems to be not so simple. The main signs of analytes' instability are inconstancy of absolute or relative areas of some chromatographic peaks (not all of them) depending on variations of analytical parameters (at first, injector or column temperatures), appearance of additional peaks, distortions of peaks' shapes, loss of separation efficiency, etc. The lack of reference RI values for any reactive compounds in contemporary mass spectrometric (**MS**) and gas chromatographic (**GC**) databases (e.g., [2]) is often due to just their instability. So-called analytical artifacts (when the results do not match the analytes containing in the samples) summarized by Middleditch [3] are often caused by the mentioned reasons.

2. Different kinds of analytes' instability complicating gas chromatographic analysis (like their classification)

As the typical examples of the manifestation of instability in the result of high reactivity of analytes (**reason I**) such semi-volatile compounds as (**3-aminopropyl)trimethoxy-** (boiling point, T_b, 194°C) and (**3-aminopropyl)triethoxysilane**

(T_b 220 ± 3°C) [4, 5] can be mentioned. It is important to note that these compounds have the normal boiling points at atmospheric pressure, but they are as active as silanization agents that can react even with glass surfaces of chromatographic syringes used for injection of samples and the silica surfaces of injector liners and chromatographic columns. Due to this reason these compounds remain uncharacterized by GC RIs up to date [2]. Another example of the same kind is so "exotic" compound as **dimethyl thionitrosamine**, **$(CH_3)_2N-N=S$** that is so unstable as the individual substance or constituent of concentrated solutions at the ambient temperature due to its easy polymerization, that it exists only in dilute solutions [6]. At the same time, this compound seems relatively stable within a chromatographic column, so that its RI on standard non-polar stationary phase has been successfully determined (992 ± 2)[1] [6].

The examples of analytes' thermal decomposition in a heated injector (**reason II**) are numerous, as well. Thus, over a dozen publications presenting RI values of **N-nitrosodiphenylamine**, **$(C_6H_5)_2N-NO$**, are known up to present, but only one of them contains the correct RI value 1865 [7], while others belong to the decomposition product – diphenylamine, **$(C_6H_5)_2NH$** (RI 1587 ± 13 [2]). For such decomposition to take place a source of active hydrogen atom is required; it can be using of hydroxyl-containing solvents, or water residues in a sample.

Sometimes the revealing of structural features of analytes' molecules responsible for their decomposition appears the important problem in organic chemistry. Thus, such heterocyclic compounds as 4-acyl-1,3,4-oxadiazolines do not differ principally from other organic compounds by their stability. Nevertheless, if these heterocycles contain no substituents in the position 2, they decompose at the injector temperatures above 150°C with formation of monoacyl hydrazones [8] in the result of decarbonylation. Within temperature range 150–190°C both initial compounds and decomposition products are detected, but above 190°C no peaks of initial analytes are registered on the chromatograms:
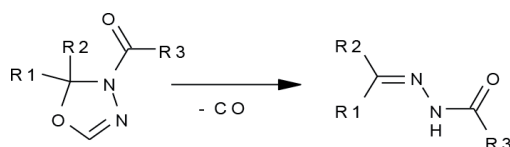
The similar decomposition is observed for substituted high reactive 3,4-dihydroformazans (trivial name "azohydrazines", but the limit of their thermal stability is less, approx. not more than 130°C [9]. The decomposition products are disubstituted hydrazones:
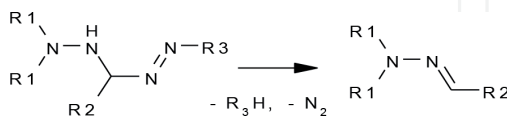
As an example of erroneous determination of retention indices in the result of thermal decomposition of analytes let us mention the RI value for trichloroacetic acid (600) on standard non-polar polydimethylsiloxane stationary phases published in Sadtler Retention Index Library [10]. It looks like obviously erroneous, but its proving appeared to be not so simple, because it requires comparing RI values for series of structural analogues of acetic acid containing up to two chlorine atoms (congeners), as it is illustrated by data in **Table 1**:

---

[1] Here and afterwards all RI values, if it is not mentioned specially, presented for standard non-polar stationary phases (polydimethyl siloxanes), i.e., RI$_{non-polar}$.

Acid congener	Structure	Boiling point, °C	RI ± s_{RI}
Acetic	CH_3CO_2H	118	624 ± 10 [2]
Chloroacetic	$ClCH_2CO_2H$	188	864 ± 12*
Dichloroacetic	Cl_2CHCO_2H	194	1048 ± 23*
Trichloroacetic	Cl_3CCO_2H	197; Extrapolated value is 194.6°C**	No experimental data; extrapolated value is 1189**

*RI values for chloro- and dichloroacetic acids are taken from author's data collection.
**T_b and RI values are extrapolated using the data for previous congeners and the recurrent algorithm [11].

Table 1.
Comparing the retention indices ($RI_{non-polar}$) for chloroacetic acids.

Known RI data for substituted acids Cl_0-Cl_2 (three points) allows approximating RIs using recurrent relation $\mathbf{RI}(n_{Cl} + 1) = a\mathbf{RI}(n_{Cl}) + b$ [11] (a = 0.767, b = 385.6), hence for trichloroacetic acid (Cl_3) RI = 0.767 × 1048 + 385.6 ≈ 1189. Thus, the value 600 from [10] is erroneous and obviously belongs to the chloroform $CHCl_3$ (reference RI value is 605 ± 4 [2]) formed in the result of decomposition of the trichloroacetic acid within heated GC injector:

$$Cl_3C - CO_2H \rightarrow CHCl_3 + CO_2$$
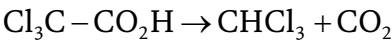
Thermal instability caused by mutual interaction of constituents of the samples in an heated injector (**reason III**) can be illustrated by features of chromatographic determination of the impurity of 1,2-propanediol (propylene glycol) in the high boiling polar aprotic organic solvent – 4-methyl-1,3-dioxolan-2-one (trivial name – propylene carbonate, T_b 242°C) [12]. The real content of propylene glycol in this solvent at the ambient temperature is strongly distorted in the result of its hydrolysis by the residual amounts of water in a heated injector during injection:
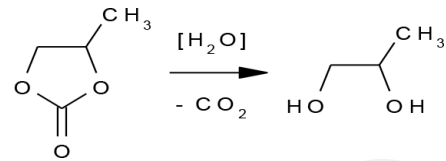
The last source of instability of analytes during GC separation – decomposition of analytes in a chromatographic column (**reason IV**) – is not equivalent to their decomposition in an injector (**reason II**). In a column analytes are usually exist under the influence of a lower temperature, but a longer time than in an injector. We cannot change the influence of column's temperature without changing the retention time (t_R), so far as these parameters are connected by the known two-parameters Antoine-like equation:

$$\log(t_R - t_0) = a / T + b \qquad (1)$$

where t_0 is hold-up time of chromatographic system, T is the absolute temperature (in Kelvins), coefficients a and b are calculated by Least Squares Methods.
The temperature dependence of the rate of first-order decomposition reaction (rate constant k) is approximated with known Arrhenius relation that is two-parameter Antoine-like equation as well:

$$\ln k = -E_a / RT + \ln A \qquad (2)$$

where E_a is activation energy for the reaction, R is universal gas constant, A is pre-exponential factor.

Thus, the influence of column's temperature on the areas of chromatographic peaks of unstable compounds is simultaneously determined by four coefficients, a, b (Eq. 1), E_a/R, and $\ln A$ (Eq. 2). Just these relationships confirm that analytes' decomposition in GC column seem to be difficult to eliminate, because if we decrease the column's temperature, we increase the analyte's residence time in the heated area. Besides the examples listed above, the decomposition inside GC column (in addition to decomposition in an injector) is valuable for diazocarbonyl compounds, decomposed with the formation of substituted ketene in the result of so-called Wolff rearrangement [13]:
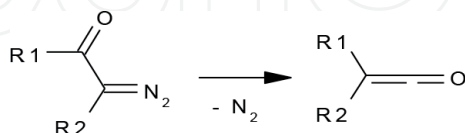
It is interesting to note that resulted ketenes are unstable compounds as well; they cannot be isolated as individual substances or components of concentrated solutions due to easy polymerization, and, hence, do not form distinct chromatographic peaks. In the result, their mass spectra were registered successfully [13], but GC RIs cannot be determined excepting the most volatile simplest member of this series – dimethyl ketene ($RI_{non-polar}$ 484, RI_{polar} 1215 [2]).

3. The influence of the decomposition of analytes in chromatographic columns on contours of chromatograms

The last mentioned mode of analytes' transformations during GC separation (**reason IV**, decomposition in a chromatographic column) is often manifested in the appearance of specific profiles of chromatograms. **Figure 1** presents a schematic image of typical chromatogram of unstable analyte "X" which is converted into decomposition product "Y" within a chromatographic column in the result of the process $X \to Y$. Usually (at least, in GC) the decomposition product "Y" is more
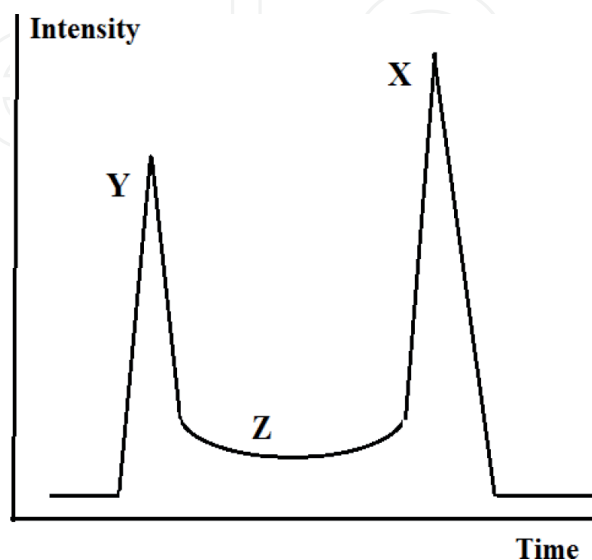

Figure 1.
Schematic image of the typical chromatogram of thermally unstable analyte "X" converted into more volatile decomposition product "Y" within chromatographic column during separation. The appearance of a diffuse zone "Z" ("train" or "plateau") between peaks "X" and "Y" is the sign of decomposition ($X \to Y$).

volatile simpler compound. The peaks of both compounds "X" and "Y" rather often can be registered on the chromatograms; and retention times of decomposition products "Y" are naturally less than those of initial analytes "X". However, besides these two regular peaks there is a specific diffuse signal between them (Z), named as "anomalous profile", "plateau", "train", "plume", or something similar (no generally accepted terminology exists). In the case of monomolecular decomposition of X, this "train" consists exclusively of decomposition product "Y".

The "idealistic" profile like that on **Figure 1** in many cases can be more or less distorted that complicates revealing the decomposition processes in a column. Some examples of such distortions are presented on Figures below. For example, decomposition of **1-diazo-4-phenylbutan-2-one**, $C_6H_5CH_2CH_2COCH=N_2$ within a column leads to the formation of "train" (Z) of ketene $C_6H_5CH_2CH_2CH=C=O$ prior the peak of initial diazocompound, but this ketene does not form a separate chromatographic peak [13].

The similar profiles of chromatograms are observed not only in cases of decomposition of analytes (irreversible reactions), but in cases of reversible interconversion of their isomeric or tautomeric forms. It should be noted that similar profiles may be observed both in GC, and HPLC. Such chromatograms were registered for keto- and enolic tautomers of 1,3-diketones (GC) [14], β-ketoesters (GC) [15, 16], *syn*- and *anti*-isomers of 2,4-dinitrophenyl hydrazones of carbonyl compounds (HPLC) [17, 18], derivatives of hydroxyquinones (HPLC) [19], and so on. The fragment of a GC–MS TIC-chromatogram illustrating the separation of keto- and enolic tautomers of ethyl acetoacetate $CH_3COCH_2CO_2C_2H_5$ is presented at **Figure 2**. The "train" observed here between the peaks of tautomers is similar to a schematic profile at **Figure 1**.

Comparing the examples mentioned we can conclude that the decomposition of unstable or reactive analytes prior to or during the chromatographic separation restricts the possibilities of this analytical technique and complicates the interpretation of results. Due to this reason any new examples of such decomposition should be reliably revealed and discussed to avoid difficulties in the subsequent data interpretation. According with this viewing, two new examples are considered here in details, namely unusual thermal instability of monoalkyl esters of benzene-1,2-dicarboxylic acid (monoalkyl phthalates) and decomposition of non-substituted hydrazones of carbonyl compounds.
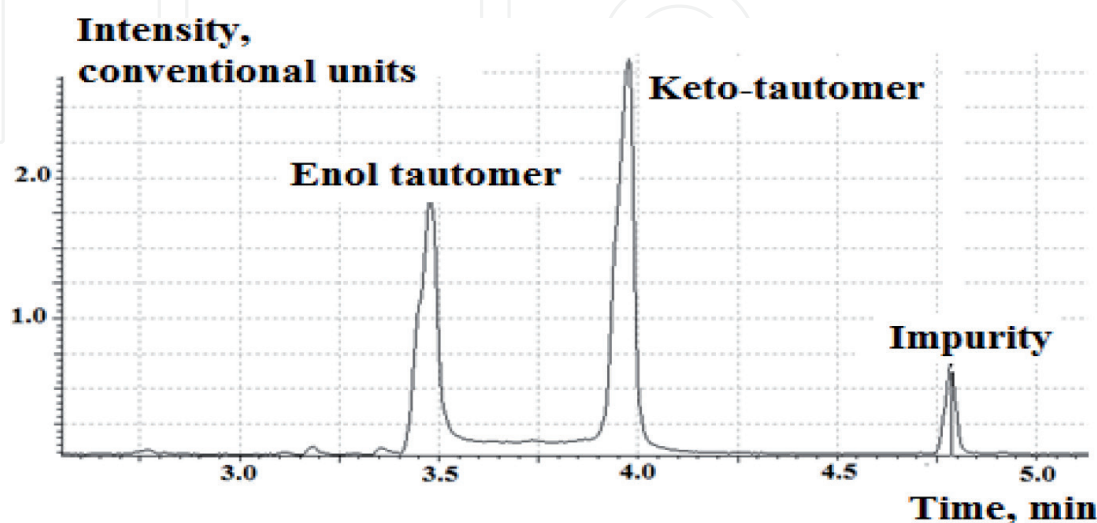

Figure 2.
*The fragment of the Total ion current (**TIC**) chromatogram (WCOT column with standard non-polar polydimethyl siloxane stationary phase) containing the peaks of keto and enol tautomers of ethyl acetoacetate **CH₃COCH₂C₂H₅**. The existence of "plateau" between peaks of tautomers confirms their interconversion within GC column during separation. Reproduced from [16] with permission.*

4. Materials and methods

Most of chemicals (alcohols, carbonyl compounds, and ethyl acetoacetate) of reagent or chromatographic grade were from "Reakhim" (Moscow, Russia). 2-Propanol and methylene chloride of chemical grade (solvents) were provided from "Vekton" (St. Petersburg, Russia). Hydrazine hydrate of reagent grade was purchased from Acros Organics, Belgium.

4.1 Preparation of reaction mixtures

Monoalkyl phthalates. Approx. 6 mg of phthalic anhydride (melting point 130–132°C, approx. 50 μmol) was added to the 2-mL portions of 1-alkanols $C_nH_{2n+1}OH$ ($n = 1$–8) and heated in the presence of catalytic amounts of phosphorous acid at the boiling point of the alcohol (for alcohols $C_1 - C_4$) or not more than 110°C (for alcohol $C_5 - C_8$) during 30 min to complete the dissolution of the phthalic anhydride. For GC–MS analysis 5 μL of reaction mixtures were diluted with 0.5–2.5 mL of methylene chloride by a factor of 100–500. All monoalkyl phthalates are characterized without their isolation from reaction mixtures. The list of RI values for monoalkyl phthalates is presented in **Table 2**.

Non-substituted hydrazones of carbonyl compounds. Hydrazine hydrate (2 mL) were mixed with 50 μL of carbonyl compounds (molar excess from 60:1 to 120:1) and 2 mL of 2-propanol at the ambient temperature. To increase the yields of azines, if necessary in some experiments, the molar ratio of hydrazine was decreased to 30:1 and 15:1. After 10 min 50 μL of obtained mixtures were diluted with 2 mL of 2-propanol, followed by addition of 2 μL of the reference *n*-alkanes mixture. All hydrazones were characterized without isolation from reaction mixtures. Besides hydrazones these mixtures contained variable amounts of azines. The list of RI values for non-substituted hydrazones and corresponding azines is presented in **Table 3**.

R in $C_6H_4(CO_2H)(CO_2R)$	M	RI [2]	RI [20]	R in $C_6H_4(CO_2H)(CO_2R)$	M	RI [2]	RI [20]
CH_3	180	1530*	1566 ± 18	*neo*-C_5H_{11}	236	1809	—
C_2H_5	194	1651	1641 ± 5	C_6H_{13}	250	2023	2029 ± 6
C_3H_7	208	1731	1734 ± 6	$-CH_2CH(C_2H_5)_2$	250	1977	—
iso-C_3H_7	208	1667	1666 ± 2	C_7H_{15}	250	2128	2132 ± 6
C_4H_9	222	1828	1849 ± 11	2-C_7H_{15}	264	—	2017 ± 10
iso-C_4H_9	222	1771	1777 ± 3	C_8H_{17}	278	2236	2250 ± 17
2-C_4H_9	222	1764*	1758 ± 3	2-C_8H_{17}	278	2143	2134
C_5H_{11}	236	1926	1929 ± 11	$-CH_2CH(C_2H_5)C_4H_9$	278	2152	—
iso-C_5H_{11}	236	—	1890 ± 7	C_9H_{19}	292	2325	—
2-C_5H_{11}	236	1842	1844 ± 2	$C_{10}H_{21}$	306	2431	—

Not experimental, but RI values evaluated using additive scheme [2] are marked with asterisk

Table 2.
Gas chromatographic retention indices of some monoalkyl phthalates on semi-standard non-polar polydimethylsiloxane stationary phases (95% methyl and 5% phenyl groups).

Carbonyl compound	RI of hydrazone	RI of azine
Acetone	750 ± 6	848 ± 3
3,3-Dimethyl- 2-butanone	936 ± 2	—
2-Hexanone	1054 ± 3	1357 ± 4 1394 ± 4*
4-Methyl-2-pentanone	970 ± 3	1249 ± 3 1298 ± 3*
2-Heptanone	1163 ± 3	1552 ± 4
3-Heptanone	1118 ± 3	1471 ± 3 1506 ± 5*
2-Octanone	1272 ± 2	1721 ± 3
Cyclopentanone	1032 ± 2	1470 ± 3
Cyclohexanone	777 ± 2	1638 ± 5
Acetophenone	1382 ± 4	2082 ± 5
4-Phenyl-2-butanone	1454 ± 3	2168 ± 5

The minor constituents with mass spectra identical to spectra of principal (E,E)-isomers tentatively belong to (E,Z)-isomers of azines.

Table 3.
Gas chromatographic retention indices of non-substituted hydrazones and corresponding azines on semi-standard non-polar polydimethylsiloxane stationary phases [24].

4.2 Instrumentation and data processing

GC–MS analyses of reaction mixtures were performed using Shimadzu QP 2010 SE gas chromatograph – mass spectrometer with electron ionization (70 eV) equipped with RTX-5 MS column of the length 30 m, internal diameter 0.32 mm, and stationary phase film thickness 0.25 μm. The conditions of analysis were as follows: temperature programming regime, initial temperature 70°C (phthalates) or 50°C (hydrazones), and ramp 6 K/min (phthalates) or 10 K/min (hydrazones) up to 200°C. Helium was used as carrier gas, flow rate was 1.8 mL/min, split ratio 1:10–1:12, injector temperatures were 200–250°C, interface and ion source temperatures were 200°C. Volumes of injected samples were 0.5 μL (phthalates) or 2 μL (hydrazones). To determine retention indices, mixtures of $C_7 - C_{20}$ n-alkanes (in different combinations) were added to the samples before analysis for determination of retention indices (RI) (calculations of linear or linear-logarithmic RIs were provided using home-made QBasic program).

Statistical data processing and plotting of results were carried out using the Origin software (versions 4.1 and 8.2). The task considered requires the specific mode of results' presentation, preferably in the form of chromatogram images. Most of original chromatograms besides the peaks of target analytes and decomposition products contain peaks of reference n-alkanes, which were required for calculation of retention indices and confirmation of the appropriate effectiveness of a column.

5. Results and discussion

5.1 Features of GC separation of monoalkyl phthalates

Contemporary GC–MS data bases (e.g., like [2]) provide important information for identification of analytes including standard electron ionization (EI) mass spectra and GC retention indices (**RI**) on standard non-polar and polar phases. However, besides

that, considering the large collection of reference data allows revealing both individual compound and their various taxonomic sub-groups (homologous series, multitudes of homologs and/or congeners, etc.) insufficiently characterized by analytical parameters up to present. One of such groups appeared to be the acidic esters of polycarboxylic acids, including alkyl esters of benzene-1,2-dicarboxylic acid (monoalkyl phthalates) that stimulated determination of their MS and GC analytical parameters [20] in comparison with the data for their much better characterized structural analogues – dialkyl phthalates. Rather unexpectedly it was found that monoalkyl phthalates appeared to be unstable at standard conditions of GC separation.

Monoalkyl phthalates can be easily synthesized from phthalic anhydride and corresponding alcohols at acid catalysis in accordance with the following scheme:
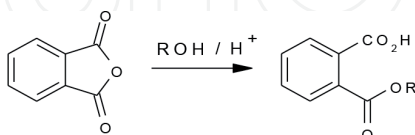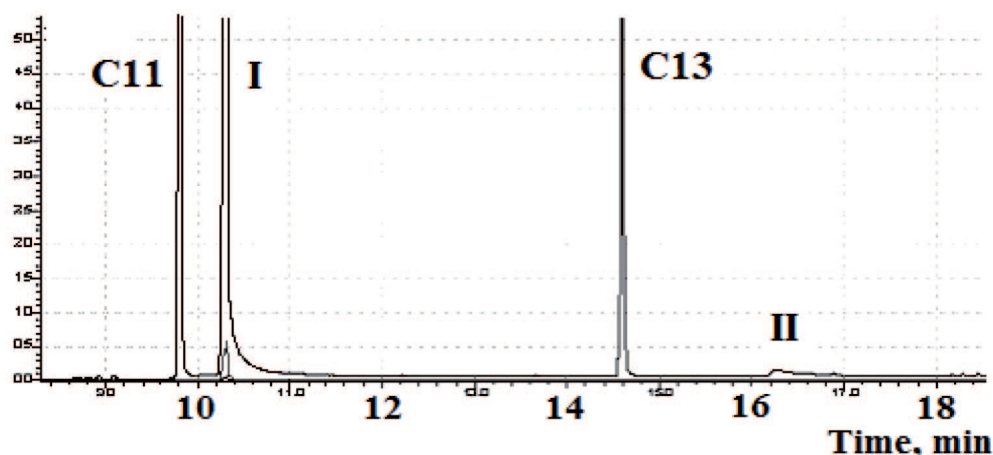

Figure 3.
The fragment of the TIC-chromatogram of reaction mixture of phthalic anhydride with methanol at the comparable scale of peak intensities. Peak (I) – Phthalic anhydride, peak (II) – Monomethyl phthalate, C_{11} and C_{13} – Peaks of reference n-alkanes. Reproduced from [20] with permission.
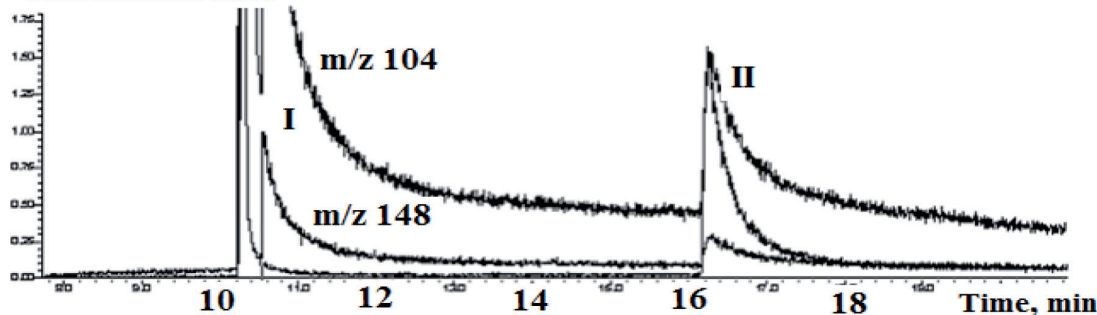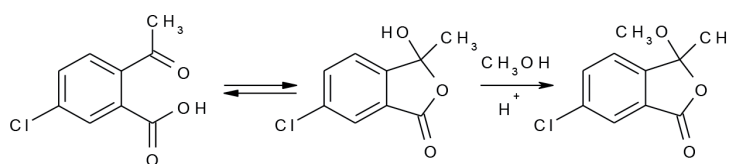

Figure 4.
The same fragment of the chromatogram of reaction mixture of phthalic anhydride with methanol as those at **Figure 3** *in SIM-regime on a larger scale; peak (I) – Phthalic anhydride, peak (II) – Monomethyl phthalate, m/z 148 – Molecular mass of phthalic anhydride, m/z 104 – Maximal signal in the mass spectrum of phthalic anhydride. Reproduced from [20] with permission.*

The large molar excess of the alcohols in the reaction mixtures allowed us to hope for a high degree of conversion of phthalic anhydride into monoalkyl phthalates. Surprisingly all the chromatograms of reaction mixtures indicate the predominant amounts of phthalic anhydride. For instance, the fragment of the TIC-chromatogram of the reaction mixture of phthalic anhydride with methanol is presented on **Figure 3**; the ratio of peak areas of anhydride (**I**) to monomethyl phthalate (**II**, RI is 1566 ± 18, retention temperature of 152°C) is approx. 100:1. To explain such an anomaly, the original chromatogram was reconstructed into SIM-mode using values m/z 148 (molecular mass of phthalic anhydride) and m/z 104 (maximal peak in its mass spectrum); the result is shown at **Figure 4**.

From **Figure 4** it is easy to notice that both signal with m/z 104 and m/z 148 besides the peak of phthalic anhydride itself indicate the second local maxima for monomethyl phthalate (**II**). However, the most important information from this SIM-chromatogram is revealing the registered "train" between peaks (**I**) and (**II**). It is the unambiguous indication of thermal instability of monomethyl phthalate at conditions of GC separation, and its main decomposition product is phthalic anhydride. The same process can be assumed for other monoalkyl phthalates having the higher boiling points than monomethyl ester, and, hence, the higher retention temperatures [20]:
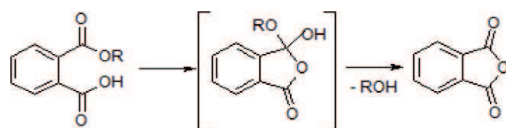
A similar process of cyclization with participation of two carbonyl groups in the *ortho*-position in benzene ring is known for chemical reaction in solution. It explains, for instance, the formation of 6-chloro-3-methoxyphthalide from 2-acetyl-5-chlorobenzoic acid [21]:

The additional independent confirmation of the thermal decomposition of monomethyl phthalate during gas chromatographic separation its mass spectrum from the database [2] should be considered. At **Figure 5** the mass spectrum of monomethyl phthalate (a) is compared with that of phthalic anhydride (b). The similarity of positions and intensities of main peaks of these spectra with m/z 104, 76, and 50 looks noteworthy. Most intensive peak of monomethyl phthalate itself belongs to the ions $[M - CH_3O]^+$ with m/z 149, but its relative intensity in mass spectrum [2] is about 40%. Moreover, the library search (reversed mode) for monomethyl phthalate [2] gives only two results with matching factor Q > 0.800, namely the same ester (another mass spectrum) with Q = 911, and phthalic anhydride (Q = 807). The similar paradox is observed even for phthalic anhydride itself; one of the results of the library search for its mass spectrum (in reversed mode) is monoethyl phthalate with Q = 854.

Nevertheless, more preferable mass spectrum of monomethyl phthalate can be obtained in the result of the accurate subtracting of background and/or overlapping signals. So as not to overload the text with figures, let us list it in the numerical form, $m/z \geq 39$ ($I_{rel} \geq 2\%$), **M** is the symbol of molecular ions:

180(2)**M**, 163(2), 150(9), 149(100) $[M - CH_3O]$, 148(10), 137(4), 136(31), 135(18), 122(5), 121(20), 118(2), 106(5), 105(52) $[M - CH_3O - CO_2]$, 104(65)
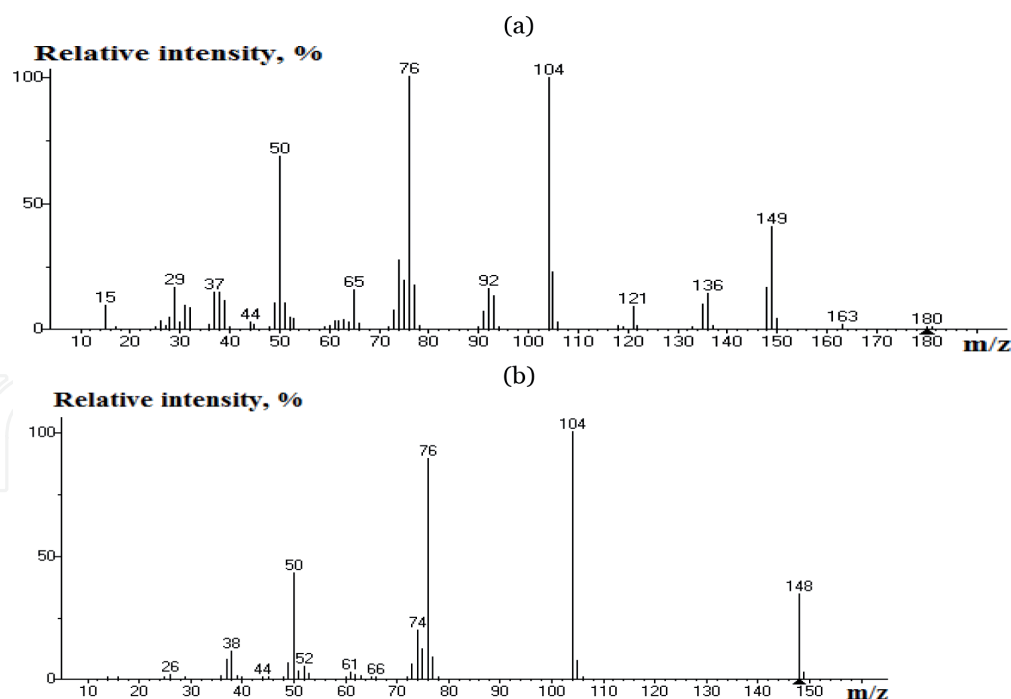
Figure 5.
Comparison of mass spectra of monomethyl phthalate (a) and phthalic anhydride (b) from database [2]. The mass spectrum of monomethyl phthalate is distorted by mass spectrum of phthalic anhydride.

$[C_7H_4O]$, 94(2), 93(29), 92(34), 91(16), 78(4), 77(33), 76(76) $[C_7H_4O -$ $CO \equiv C_6H_4]$, 75(14), 74(21), 73(5), 71(3), 66(5), 65(37), 64(5), 63(5), 59(3), 58(2), 57(2), 53(3), 52(6), 51(16), 50(46) $[C_4H_2]$, 49(4), 44(3), 43(3), 41(2), 39(24).

As it can be seen, this mass spectrum strongly differs with that from database [2]; the maximal peak appears to be m/z 149 as it should be for esters of phthalic acid [22]. However, the presence of the signals with m/z 104, 76, and 50 do not allow the excluding it's at least partial decomposition in a GC column or at any point between column and ion source of mass spectrometer.

The instability of monoalkyl phthalates – the products of partial hydrolysis of dialkyl phthalates – widely used plasticizers of polymeric compositions – permits us to suggest the novel interpretation of endocrinic toxicity of these esters. Their decomposition in small extent can take place not only at the heating, but at the ambient conditions, as well. The product of such decomposition – phthalic anhydride – is an active acylation reagent which can react with some targets inside the living cells (e.g., peptides or nucleic acids) [20].

5.2 Anomalous chromatographic properties of non-substituted hydrazones of carbonyl compounds

The considering of database [2] allows revealing another series of simple organic compounds that are characterized in enough extent neither mass spectra, nor GC retention indices. It is the products of the nucleophylic addition of hydrazine to carbonyl compounds – non-substituted hydrazones. These compounds are used in practice of organic synthesis since 19th century:
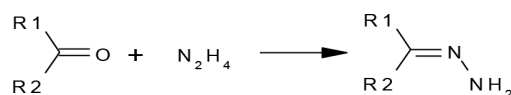
Such hydrazones can be synthesized from carbonyl compounds in one stage; they are intermediates in so-called Wolff-Kishner reduction of carbonyl
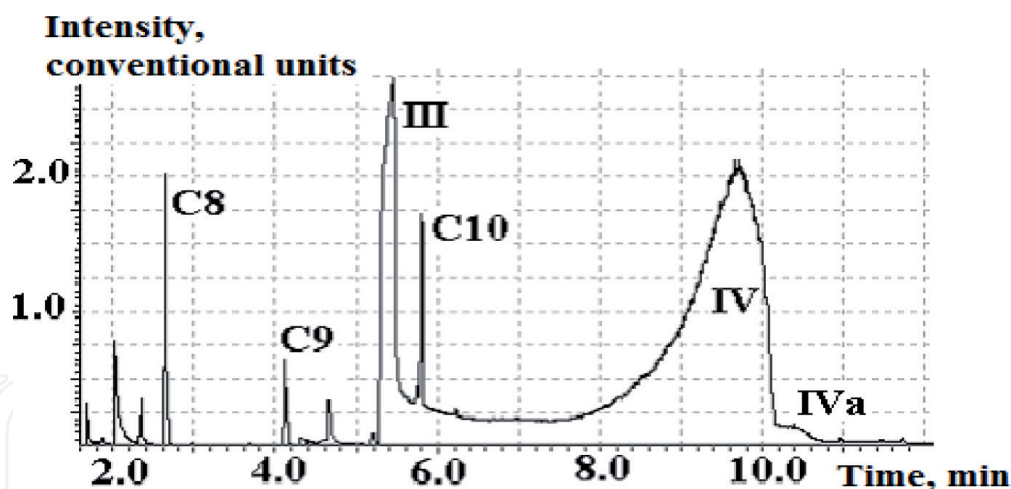
Figure 6.
The fragment of the TIC-chromatogram of reaction mixture of 4-methyl-2-pentanone with hydrazine hydrate; peak (III) – 4-methyl-2-pentanone hydrazone, peak (IV) – 4-methyl-2-pentanone azine, $C_8 – C_{10}$ – Reference n-alkanes. Reproduced from [24] with permission.

compounds into hydrocarbons with the same carbon skeletons [23]. Under such circumstances it seems nearly paradoxical why these synthetically important compounds remained not characterized both by mass spectra and by gas chromatographic analytical parameters until last time. The database [2] contains mass spectrum and RI value for only one simplest member of this series – acetone hydrazone $(CH_3)_2C=N-NH_2$.

Such inconsistency explains the necessity to carry out GC–MS analysis of reaction mixtures of carbonyl compounds with hydrazine hydrate [24]. Surprisingly, instead of "normal" (more or less sharp) chromatographic peaks of hydrazones the very "blurry" signals were recorded for them, as it can be seen from fragment of TIC-chromatograms of the reaction mixtures of hydrazine hydrate with 4-methyl-2-pentanone (**Figure 6**). The chromatograms of reaction mixtures of other carbonyl compounds look similar (see ref. [24]). Any attempts to improve the shapes of chromatograms by varying separation conditions or by dilution of samples remained unsuccessful.

All the chromatograms contain two diffuse peaks with variable relative intensities connected by "trains" between them. Strong broadening the peaks explains us the low accuracy of determining the retention indices of reaction products (and their low interlaboratory reproducibility, as well). From mass spectrometric data it is easy to conclude that molecular masses of the first eluted peaks correspond to non-substituted hydrazones, while those of the latter peaks – to azines of carbonyl compounds – stable products of the bimolecular decomposition of non-substituted hydrazones:
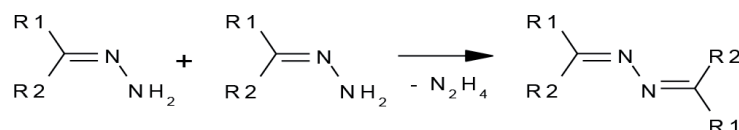
Besides this way of formation, more stable azines are the "normal" by-side reaction products even at the large excess of hydrazine. Mass spectra registered in various points between hydrazones and azines indicate that the "trains" between them are formed both by hydrazones and azines in variable proportions. It is the principal difference of the nature of such "trains" for mono-molecular decomposition processes in a GC column, when these areas are formed by decomposition products only.

12

The decomposition of just non-substituted hydrazones in a GC column is confirmed by analysis of reaction mixtures containing solely azines. For instance, the chromatograms of the reaction mixtures of cyclohexanone with hydrazine hydrate in 10 minutes after mixing the reagents and after one week storage this sample at the ambient temperature look rather different. On the first chromatogram the intensive peak of hydrazone is observed, whereas prior to the peak of azine there is the "train" confirming decomposition process in a GC column. One week later when the sole reaction product appeared to be the azine; the "train" before its peak is disappeared completely.

The decomposition of monoalkyl phthalates considered in the Section 3.1 like other known decomposition processes in a chromatographic column is the first order reactions (monomolecular). On the contrary, the decomposition of non-substituted hydrazones with formation of azines is the first revealed example of second order (bimolecular) reaction in gas chromatographic column. It should be specially noted that the observed features of chromatograms ("trains" or "plateau") in the case of bimolecular decomposition of non-substituted hydrazones are the same, as those in the cases on monomolecular reactions.

5.3 Decomposition followed by secondary chemical reactions in GC column

After examples presented above we can consider the most unusual example of analytes' conversion in a GC column. **Figure 7** contains the fragment of the TIC-chromatogram of aromatic carbonyl compound – acetophenone – with hydrazine hydrate. Similarly to the previous examples we observe the peaks of acetophenone hydrazone (**IX**, t_R approx. 11.5 min), acetophenone azine (**X**, t_R approx. 19.0 min) connected by a "train" between them (zone **Z1**), as well the peak of initial acetophenone (t_R approx. 7.5 min). However, besides the expected zone **Z1**, the second anomalous area **Z2** is observed prior to the peak of hydrazone (**IX**). Mass-spectra registered in the different points of this area **Z2** indicated that it composed exclusively of acetophenone hydrazone with the following mass spectrum in the numerical form, $m/z \geq 39$ ($I_{rel} \geq 2\%$):

135(10), 134(100)**M**, 133(21), 120(4), 119(42) [M – CH$_3$], 118(5), 117(20) [M – NH$_3$], 104(3), 103(12), 102(3), 93(9), 92(10), 91(4), 90(2), 89(2), 79(2), 78(10), 77(88) [C$_6$H$_5$], 76(7), 75(3), 74(3), 66(4), 65(6), 63(4), 57(9), 56(5), 52(3), 51(19).

The paradox observed is the follows: the area **Z2** corresponds to the range of retention times which are less than retention time of "normal" acetophenone hydrazone. In other words, *some part of acetophenone hydrazone is eluted from GC column*
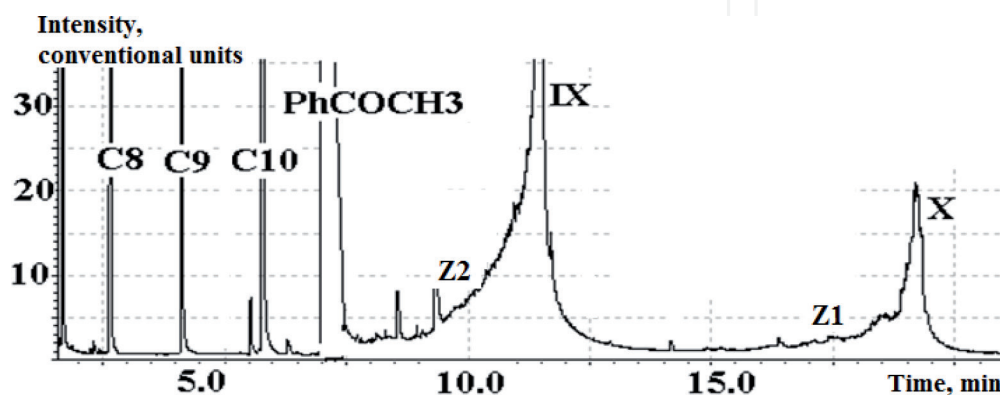

Figure 7.
*The fragment of the TIC-chromatogram of the reaction mixture of acetophenone with hydrazine hydrate; peak (**IX**) – Acetophenone hydrazone, peak (**X**) – Acetophenone azine, C$_8$ – C$_{10}$ – Peaks of reference n-alkanes. Reproduced from [24] with permission.*
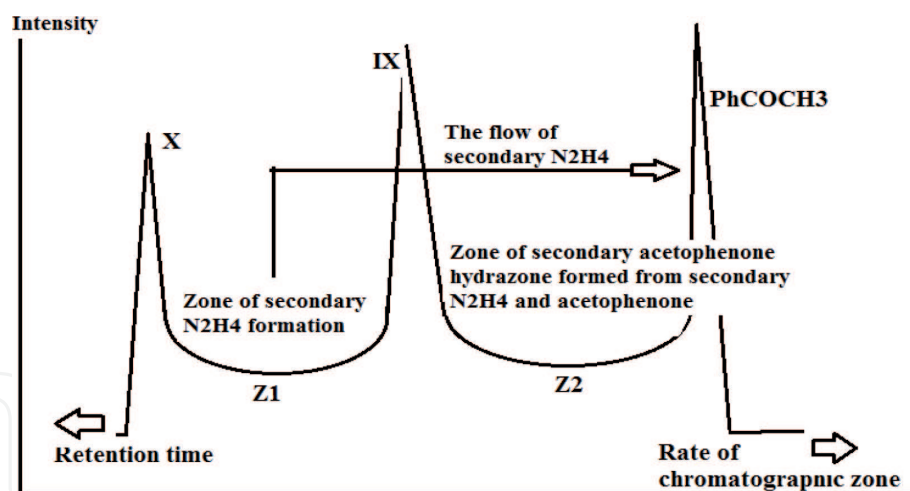
Figure 8.
The scheme of formation of two "trains" on chromatograms. Zone Z_1 – Area of acetophenone hydrazone (IX) decomposition with the formation of acetophenone azine (X) and secondary hydrazine; zone Z_2 – Area of interaction of secondary hydrazine with residues of initial acetophenone in reaction mixture with formation of secondary acetophenone hydrazone been eluted before "normal" acetophenone hydrazone.

"before" acetophenone hydrazone (!). Even the very formulation of this effect looks nearly paradoxical. It is the first known example of so anomalous chromatographic behavior of analytes.

To explain this anomaly let us reconstruct schematically the TIC-chromatogram of reaction mixture of acetophenone with hydrazine hydrate in the reversed scale, like it is shown at **Figure 8**. At this scheme an increase in retention times corresponds to a direction to the left, while an increase in the rate of chromatographic zones within column – to a direction to the right. The decomposition of acetophenone hydrazone (**IX**) with formation of acetophenone azine (**X**) (according with the scheme of reaction above) is occurs in zone **Z1** and it is accompanied by formation of secondary hydrazine that is most volatile constituent comparing with other reaction products (T_b 114°C). Thus, the rate of chromatographic zone of secondary hydrazine exceeds the rates of chromatographic zones of other components of reaction mixtures. This hydrazine zone can "catch up" the zone of initial acetophenone and reacts with it, that leads to the formation of the "train" of secondary acetophenone hydrazone (**Z2**) located *between* the peaks of acetophenone itself and the "normal" peak of acetophenone hydrazone (i.e., before it). Of course, this reaction of nucleophylic addition of hydrazine to the carbonyl compound proceeds not in the gaseous, but in the condensed phase, namely in the stationary phase film.

Despite of the highly "exotic" character of the examples considered, the knowledge of them seems to be useful for better revealing the analogous situations in routine chromatographic practice.

5.4 Criterion of applicability of GC / GC-MS analysis for thermally unstable compounds

Obviously, thermal instability of analytes seems to be the principal restriction of gas chromatographic separation of highly reactive compounds, thus we cannot eliminate it for objective reasons. However, in some cases we can evaluate the temperature limits of chromatographic columns, which should not be exceeded when analyzing certain unstable analytes.

It is known that the low boiling simplest homologs of thermally unstable compounds can often be distilled at ambient conditions without their thermal decomposition and, therefore, they are characterized by "normal" boiling point at

atmospheric pressure (T_b, °C). The existence of boiling point means the possibility of gas chromatographic analysis of such compounds using contemporary fused silica WCOT columns of high inertness. Heating the higher members of the series to their boiling points at atmospheric pressure appeared to be impossible because of their decomposition. In practice of organic synthesis, distillation of such compounds under reduced pressure is used. Thus, we can select the boiling point of simplest homolog of the series under consideration at atmospheric pressure as the GC and/or GC–MS analyses temperature limit (T_{lim}) for other members of these homologous series. If GC separation is carried out not in isothermal, but in temperature programming conditions, instead of fixed column's temperature we must operate with so-called retention temperature (T_R) which should not exceed T_{lim} value:

$$T_R = T_0 + rt_R \qquad (3)$$

where T_0 is the initial temperature, t_R – retention time, r – ramp (centigrade per time unit).

To illustrate this criterion, let us consider the boiling point of the series of thermally unstable alkyl azides, **R-N₃** in comparison with data for dialkyl diimides **R-N=N-R** (**Table 4**). In both series these data are available for homologs with R $\leq$ C₅H₁₁. Boiling points of homologs with R $\geq$ C₆H₁₃ at atmospheric pressure remain unknown at present due to instability of such compounds. Therefore, GC and/or GC–MS analysis of alkyl azides should be possible up to their retention temperature (T_R) not exceeding approx. $T_{lim} \approx$ 130–135°C (it has been confirmed experimentally [25]), and approx. $T_{lim} \approx$ 180°C for dialkyl diimides. This conclusion is confirmed by experimental RI values known just for some homologs of these series. On the other hand, within the alkyl hypochlorites series, **R-OCl**, T_b values are known only for members with R = CH₃ (9.2°C), R = C₂H₅ (27–36°C), and R = *tert*-C₄H₉ (77–78°C), meaning that T_{lim} for this series is not more than approx. 75–80°C. However, it is enough for separation of simplest homologs and determining their RI values, namely 502 (ethyl hypochlorite) and 605 (*tert*-butyl hypochlorite).

Within the series of aliphatic diazocarbonyl compounds with structural fragment -**CO-CHN₂**, ethyl diazoacetate, **N₂CHCO₂C₂H₅**, is one of the most often used reagents. Hence, the physicochemical properties available for this compound (including T_b 140–143°C) appear to be the most reliable comparing with data for other homologs [26]. We could not find in the literature the normal T_b values for higher alkyl diazoacetate homologs (with R $\geq$ C₂H₅) just due to their instability, but we can conclude that GC and/or GC–MS analysis of other diazocarbonyl

| R in R-N₃	T_b, °C	RI$_{non-polar}$	R in R-N=N-R	T_b, °C	RI$_{non-polar}$
CH₃	20–21	457	CH₃	1.5–2.0	396 ± 5
C₂H₅	48–50	543	C₂H₅	59	563 ± 4
C₃H₇	77.5–78	634	C₃H₇	113–115	756 ± 6
C₄H₉	106.5	746	C₄H₉	145.145.5	867 ± 2
C₅H₁₁	130–135	845	(C₂H₅)₂CH	182	1052 ± 16
$n \geq 6$	no data	no data	$n \geq 6$	no data	no data
T_{lim}	~ 130–135			~ 180	

Table 4.
Boiling points of some alkyl azides R-N₃ and dialkyl diimides R-N=N-R at atmospheric pressure and their GC retention indices on standard non-polar polydimethylsiloxane stationary phases.

compounds should be possible up to a temperatures of GC column approx. $T_{lim} \approx 140°C$. The applicability of this criterion has been verified during analysis of aryl substituted diazocarbonyl compounds [13]. For instance, such analyte as 1-diazo-4-phenylbuten-2-one, $C_6H_5CH_2CH_2COCHN_2$, was analyzed under conditions ensuring
its retention temperature 145°C, slightly exceeding $T_{lim} \approx 140°C$. Exceeding the limit naturally leads to the appearance of a "plume" prior to the chromatographic peak of diazocompound that belongs to decomposition product, namely 4-phenyl-2-buten-1-one, $C_6H_5CH_2CH_2CH=C=O$.

Thus, the sense of the chemical criterion for GC and/or GC–MS analysis of thermally unstable compounds is revealing their simple homologs for which the reference information on their normal boiling points (at atmospheric pressure) is available. The general recommendation to avoid the decomposition of unstable analytes in a GC column is not to exceed the column's temperature above this limiting value.

The criterion considered can be re-formulated, if necessary. If some homologs of any class of potentially unstable compounds indicate stability at sufficiently high retention temperatures, we can consider these T_R values as the limiting T_{lim} values for other homologs of the same series, or their structural analogues. Such viewing allows evaluating the really anomalous thermal stability of organic hydroperoxides, **R-OOH**, and, especially, peroxides, **RO-OR**.

Improvements of contemporary fused quartz capillary columns in GC (increasing of their inertness) permits us to use them, for example, for separation of obviously unstable hydroperoxides formed from monoterpene hydrocarbons in plant essential oils [27], cyclohexyl- and cycloheptylhydroperoxides and corresponding dicyclohexyl- (**I**) and dicycloheptylperoxides (**II**) [28]. Most exotic structures with peroxide fragments which can be separated using GC without decomposition are 3,3,6,6-tetramethyl-1,2,4,5-tetraoxocyclohexane (trivial name "diacetone diperoxide", **III**), 3,3,6,6,9,9-hexamethyl-1,2,4,5,7,8-hexaoxocyclononane ("triacetone triperoxide", **IV**), and even 3,3,6,6-tetrapropyl-1,2,4,5-tetraoxocyclohexane (**V**) [29]:
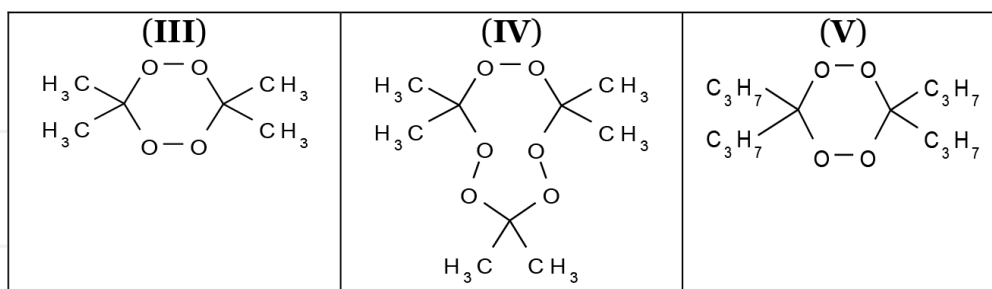

The most notable are the high retention temperatures of mentioned compounds: T_R value for dicyclohexylperoxide (**I**) is approx. 129°C, for dicycloheptylperoxide (**II**) ~ 180°C, for compound (**IV**) – 110-160°C (in different regimes), and for compound (**V**) – 185-260°C (!). This corresponds to the possibility of peroxides separation in almost any temperature regimes without risk of thermal decomposition of analytes and no needs for special control of separation conditions.

This conclusion was applied in the analysis of the unusual impurity found in the sample of benzyl alcohol $C_6H_5CH_2OH$ [30] with the retention index 1894 ± 10 (semi-standard non-polar stationary phase RTX-5 MS) and the following mass spectrum, $m/z \geq 39$ ($I_{rel} \geq 2\%$), **M** is the symbol of molecular ions:

230(1)**M**, 213(2), 198(2), 197(8), 107(14), 105(6), 92(23), 91(100), 79(3), 77(6), 51(2), 39(2).

Attempts to identify this compound using the database [2] appeared to be unsuccessful. Nevertheless, the detailed interpretation of this mass spectrum together with GC retention index permits us to establish its structure unambiguously. The maximal signal with m/z 91 confirms the presence of benzyl fragment in the molecule, the peak with m/z 213 belongs to the ions $[M - 17] \equiv [M - OH]^+$, and the peak with m/z 197 – to the ions $[M - 33] \equiv [M - OOH]^+$. Combining the available chemical and spectral information, we can attribute solely the structure of dibenzyl ether hydroperoxide for this impurity [30]:
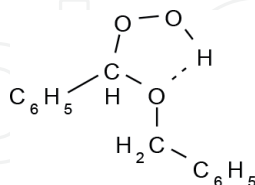
The existence and stability of such hydroperoxide seem rather unusual. At first, the presence of two functional groups (one of them with active hydrogen) at one carbon atom looks like very "exotic" structure of unstable hydroperoxide of semiacetal. The second feature is high retention temperature of this impurity (approx. 190°C). Nevertheless, in accordance with criterion mentioned above so high T_R value does not restrict GC separation of this compound without its thermal decomposition.

It is interesting to note that hydroperoxide of similar structure, $C_2H_5OCH(OOH)CH_3$, formed from diethyl ether, $(C_2H_5)_2O$, was detected by gas chromatographic analysis (RI 794) without its decomposition [31].

6. Conclusions

Two new examples of thermal decomposition of analytes during GC separation within a gas chromatographic column were revealed and considered. First of them is monomolecular decomposition of monoalkyl phthalates with the formation of phthalic anhydride. The second process – decomposition of non-substituted hydrazones of carbonyl compounds – seems like the first example of bimolecular reactions of analytes in a GC column. However, the visual manifestations of all these processes on the chromatograms are identical to the manifestations of interconversion of isomers, for example, keto-enol transformations of ethyl ester of acetoacetic acid in a GC column. The profiles of chromatograms in most cases contain a peak of initial analyte, a peak of a product of its decomposition or isomerization, and more or less expressed diffused "plateau" or "train" between them. Besides that, the first example of secondary chemical reaction in a GC column, when the decomposition by-product reacts with other constituents of samples, is revealed for hydrazones of alkyl aryl ketones.

Acknowledgements

Author details

Igor G. Zenkevich
St. Petersburg State University, Institute for Chemistry, Universitetskii Prospect 26,
St. Petersburg 198504, Russia

*Address all correspondence to: izenkevich@yandex.ru

IntechOpen

References

[1] Ivanova N.T., Frangulyan L.A. GC analysis of unstable and reactive compounds. Khimia Publ., Moscow, 1979. (In Russian).

[2] The NIST 17 Mass Spectral Library (NIST17/2017/EPA/NIH). Software/ Data Version (NIST17); NIST Standard Reference Database, Number 69, June 2017. National Institute of Standards and Technology, Gaithersburg, MD 20899: http://webbook.nist.gov (accessed: August 2020).

[3] Middleditch B.S. Analytical Artifacts: GC, MS, HPLC, TLC, and PC. J. Chromatogr. Library. Vol. 44. Elsevier, Amsterdam, 1989.

[4] Jain A., Hirata G.A., Farias M.H., Castillon F.F. Synthesis and characterization of (3-aminopropyl) trimethoxy-silane (APTMS) functionalized Gd2O3:Eu(3+) red phosphor with enhanced quantum yield // Nanotechnology. 27(6) (2016) 065601. doi: 10.1088/0957-4484/27/6/065601.

[5] Howarter J.A., Youngblood J.P. Optimization of silica silanization by 3-aminopropyltriethoxysilane // Langmuir. 22(26) (2006) 11142-11147. doi: 10.1021/la061240g.

[6] Zenkevich I.G., Ul'yanov A.V., Golub S.I., Buryak A.K. Chromato-graphic component of identification of the transformation products of 1,1-dimethylhydrazine in the presence of sulfur // Rus. J. General Chem. 84(6) (2014) 1102-1110. doi: 10.1134/S1070363214060097.

[7] Gough, T.A.; Webb, K.S. The trace detection of some non-volatile nitrosamines by combined gas chromatography and mass spectrometry // J. Chromatogr. 95 (1974) 59-63. doi: 10.1016/s0021-9673(00)84095-9.

[8] Zenkevich I.G., Yandovskii V.N. Chromato mass-spectrometric characterization of substituted 4-acyl-1,3,4-oxadiazolines // Chem. Heterocycl. Comp. 5 (1984) 498-505.

[9] Ioffe B.V., Kuznetsov M.A., Chernyshov V.A., Kuznetsova L.M., Zenkevich I.G. Heminal azohydrazines – the new class of organic compounds // Rus. J. Org. Chem. 12(11) (1976) 2273-2281. (In Russian).

[10] The Sadtler Standard Gas Chromatography Retention Index Library. Vols. 1-4. Sadtler Division, Bio-Rad Laboratories, Philadelphia, PA, 1986.

[11] Zenkevich I.G. Application of Recurrent Relationships in Chromatography // J. Chemometrics 23 (2009) 179-187. doi: 10.1002/cem.1214.

[12] Zenkevich I.G., Bystrova G.I. Quantitative analysis of mixtures unstable at the conditions of chromatographic separation. Determination of propylene glycol impurity in propylene carbonate // J. Analyt. Chem. (Rus.). 40(9) (1985) 1686-1693.

[13] Kornilova T.A., Ukolov A.I., Kostikov R.R., Zenkevich I.G. A simple criterion for gas chromatography/mass spectrometric analysis of thermally unstable compounds, and reassessment of the by-products of alkyl diazoacetate synthesis // Rapid Commun. Mass Spectrom. 27(3) (2013) 461-466. doi: 10.1002/rcm.6457.

[14] Masur M., Grutzmacher H.-F., Munster H., Budzikiewicz H. Mass spectrometric fragmentation of the tautomers of 1,3-diketones // Org. Mass Spectrom. 22 (1987) 493-500.

[15] Allegretti P.E., Schiavoni M.M., Di Loreto H.E., Furlong J.J.P., Della Vedova C.O. Separation of keto-enol tautomers in β-ketoesters: a gas

chromatography – mass spectrometric study // J. Mol. Struct. 560 (2001) 327-335.

[16] Zenkevich I.G., Lukina V.M. Characteristic features of the gas chromatographic separation of tautomers of ethyl acetoacetate // J. Phys. Chem. A. 2020 94(6) 1214-1223. doi: 10.1134/S0036024420060357.

[17] Trapp O., Shelle R., Marriott P., Schurig V. Simulation of elution profiles for two-dimensional dynamic gas chromatographic experiments // Anal. Chem. 75(17) (2003) 4452-4461. doi: 10.1021/ac0301144.

[18] Practical Gas Chromatography. Eds. W. Engewald, K. Dettmer-Wilde. Springer-Verlag. Berlin, 2014. 902 p.

[19] Shinde Y., Sproules S., Kathawate L., Pal S., Konkimalla B., Salunke-Gawali S. Separation and isolation of tautomers of 2-hydroxy-4-naphthoquinone-1-oxime derivatives by liquid chromatography: antiproliferative activity and DFT studies // J. Chem. Sci. 2014 126(1) 213-225.

[20] Zenkevich I.G., Fakhretdinova L.N. Thermal instability of monoalkyl esters of phthalic acid during their gas chromatographic separation // Analytics & Control. 19(2) (2015) 175-182. doi: 10.15826/analitika.2015.19.2.013. (In Russian).

[21] Khaibulova T.S., Boyarskaya I.A., Larionov E., Boyarskiy V.P. Cobalt-catalysed methoxycarbonylation of substituted dichlorobenzenes as an example of a facile radical anion nucleophylic substitution in chloroarenes // Molecules, 19 (2014) 5876-5897. doi: 10.3390/molecules19055876.

[22] Hamming M.C., Foster N.G. Interpretation of mass spectra of organic compounds. Academic Press. New Yotk. 1979. 694 p.

[23] Kuethe J.T., Childers K.G., Peng Z., Journet M., Humphrey G.R., Vickery T.,

Bachert D., Lam T.T. A practical kilogram-scale implementation of the Wolff-Kishner reduction // Org. Process Res. Development. 13(3) (2009) 576-580. doi: 10.1021/op9000274.

[24] Zenkevich I.G., Podol'skii N.E. Revealing compounds unstable during gas chromatographic separation. Non-substituted hydrazones of carbonyl compounds // Analytics & Control. 21(2) (2017) 125-134. doi: 10.15826/analitika.2017.21.2.002. (In Russian).

[25] Akhlaghinia B., Samiei S. A novel and highly selective conversion of alcohols, thiols, and silyl ethers to azides using the 2,4,6-trichloro[1,3,5]triazine / n-Bu4NN3 system // J. Braz. Chem. 18(7) (2007) 1311-1315. doi: 10.1590/S0103-50532007000700003.

[26] Clark J.D., Shah A.S., Peterson J.C. Understanding the large-scale chemistry of ethyl diazoacetate via reaction calorimetry // Thermochimica Acta. 392-393 (2002) 177-186. doi: 10.1016/S0040-6031(02)00100-4.

[27] Rudback J., Ramzy A., Karlberg A.-T., Nilsson U. Determination of allergenic hydroperoxides in essential oils using gas chromatography with electron ionization mass spectrometry // J. Sep. Sci. 37(8) (2014) 982-989. doi: 10.1002/jssc.201300843.

[28] Wierzchowski P.T., Zatorski L.W. Determination of cyclo C_6 and C_7 peroxides and hydroperoxides by gas chromatography // Chromatographia. 51(1-2) (2000) 83-86.

[29] Barreto G.P., Canizo A.I., Eyler G.N. Use of retention data as the first step in the identification of cyclic organic peroxides in temperature-programmed gas chromatography // Chromatographia. 63(5-6) (2006) 261-266. doi: 10.1365/s10337-006-0716-y.

[30] Zenkevich I.G. Special features of gas chromatographic determination

of dibenzyl ether hydroperoxide
admixture in benzyl alcohol // Rus. J.
General Chem. 86(9) (2016) 2016-2021.
doi: 10.1134/S1070363216090061.

[31] Delort E., Jaquier A. Novel terpenyl
esters from Australian finger lime (*Citrus
australasica*) peel extract // Flav. Fragr. J.
24(3) (2009). doi: 10.1002/ffj.1922.