

Retention of Oximes of Aromatic Carbonyl Compounds Under Conditions of Reversed Phase HPLC

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Abstract—Anomalies in the chromatographic retention of sorbates in reversed phase HPLC are often attributed to variations in their mechanisms of retention. However, an equally important reason seems to be a change in the chemical nature of sorbates due to interaction with components of the eluent. Chromatographic properties of several oximes of aromatic carbonyl compounds in high-performance reversed phase liquid chromatography are characterized, including their retention indices and results from recurrent approximation of the dependences of sorbate retention on the concentration of the organic component of the eluent. Such approximation allows us to identify considerably more anomalies in the retention times than other means. Chromatographic information is supplemented by spectral parameters, specifically relative optical densities $A_{\text{rel}} = A(\lambda_1)/A(\lambda_2)$. Compounds that are stable under conditions of separation are found in the series of oximes, along with examples of reversible hydration (oximes of 2-methoxy- and 3,4-dimethoxybenzaldehydes) and irreversible hydrolysis (oximes of 2- and 4-hydroxybenzaldehydes, acetophenone) with the formation of the corresponding aldehydes. It is shown that coefficients of the dependence of retention indices on the concentration of the organic component of the eluent for aldehydes mainly satisfy inequality $dRI/dC > 0$, and they are usually negative for their oximes. The differences between indices of retention of retention $\Delta RI = RI(\text{oxime}) - RI(\text{aldehyde})$ in high-performance reversed phase (RP) liquid chromatography are consequently not constant. Instead, they fall as the concentration of methanol in the eluent rises.

Keywords: oximes of aromatic carbonyl compounds, high-performance reversed phase liquid chromatography, retention indices, recurrent approximation of retention times

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INTRODUCTION

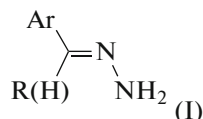
The need and expediency of characterizing organic compounds using gas chromatographic retention indices (RI) [1] is currently beyond doubt, as is confirmed by the constant expansion and improvement of reference information arrays that include such data. For example, the latest version (2023) of the NIST database [2] contains about 492 thousand values of such indices of retention for approximately 153 thousand compounds. Such arrays are useful not only for identifying the objects represented in them, but also for the purpose of identifying previously uncharacterized compounds missing from databases.

The concept of retention indices is much less popular in reversed phase high-performance liquid chromatography (RP HPLC), despite known examples of its effective application [3, 4]. This is because these parameters in this mode of separation are influenced by many more factors (e.g., composition and eluent pH) than in gas chromatography. This reduces their interlaboratory reproducibility and, combined with a narrower range of variations, makes them less informative. In addition, numerous interactions between

sorbates and eluent components are possible, including shifts in acid–base equilibria, tautomeric transformations, the reversible formation of hydrates, irreversible hydrolysis and other processes. Such instability of the analyzed compounds complicates identifying patterns of their behavior in RP HPLC (including the determination of RI), so examples of it deserve detailed characterization.

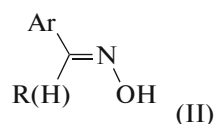
If we talk about the chemical nature of sorbates for which interaction with the components of eluents in RP HPLC is most likely, they are mainly the most polar compounds (hydration) and others that can form as a result of condensation reactions with the elimination of water (hydrazones, oximes, semicarbazones, acetals, ketals, and others). Since eluents in RP HPLC contain water, its presence can lead to reverse hydrolysis reactions of such compounds, especially in an acidic environment (pH < 7). If confirming the rapid irreversible hydrolysis of such sorbates usually does not create difficulties (as a result of, e.g., comparing their parameters of retention with the parameters of the expected products of hydrolysis), identify-

ing hydrolysis during separation is a more complicated task. Similar features have been noted in, e.g., the characterization of non-substituted hydrazones of aromatic carbonyl compounds:



Hydrazones of alkyl aromatic ketones are stable under conditions of separation, while hydrazones of aromatic aldehydes are prone to partial or complete hydrolysis in the eluent [5]. Since such hydrazones are thermally unstable under conditions of gas chromatographic separation [6], this creates serious problems in analyzing compounds of this class.

The instability of some unsubstituted hydrazones under conditions of RP HPLC forces us to consider in more detail the possibilities of applying this means of separation to oximes, their closest structural analogs:

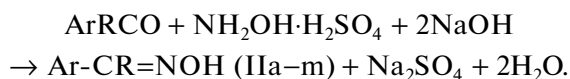


As a result of comparing the chemical properties of these compounds, it was suggested in [7] that oximes are generally more resistant to hydrolysis than unsubstituted hydrazones, but the possibility of this process occurring under conditions of RP HPLC cannot be completely excluded. The aim of this work was to test this assumption in determining the indices of retention of oximes of aromatic carbonyl compounds.

Until now, the main way of characterizing the chromatographic behavior of sorbates in RP HPLC was to establish the dependence of their retention times (or related variables, e.g., coefficients of capacity, factors and retention volumes) on the content of organic components of eluents, $t_R(C)$ [8–13]. Such characterization was often done in order to select the optimum conditions for the separation of sorbates. Cyclohexanone oxime [14], oximes of several aromatic aldehydes [15], and some antibiotics [16, 17] are among the group of these compounds that have been characterized in this way. An unusual characteristic of the $t_R(C)$ dependences (with minima) has been revealed for oximes containing pyridinium cations [18]. In [19], we were able to separate prototropic tautomers for 2-hydroxy-4-naphthoquinone-1-oxime derivatives. However, systematic compounds of this class have generally not been characterized. For example, neither gas chromatographic RI values [2] nor similar data in RP HPLC are known for oximes of the simplest aromatic aldehyde, benzaldehyde.

EXPERIMENTAL

Oximes of aromatic carbonyl compounds have been synthesized by reacting hydroxylamine sulfate (Reakhim, Moscow) in alkaline media with such substituted benzaldehydes as (a) 2-methyl- (Lancaster), (b) 4-methyl-, (c) 2-hydroxy- (Aldrich, United States), (d) 4-hydroxy-, (e) 2-methoxy- (Fluka, United Kingdom), (f) 4-methoxy- (Reakhim, Kyiv plant), (g) 3-hydroxy-4-methoxy (vanillin, Ferak Berlin, Germany), (h) 4-hydroxy-3-methoxy (isovanillin; Janssens Chimica, Belgium), (i) 3,4-dimethoxy (veratric aldehyde; Acros, Belgium), alongside ketones $\text{C}_6\text{H}_5\text{COC}_n\text{H}_{2n+1}$ with $n = 1-3$: (j) acetophenone, (k) propiophenone, and (l) butyrophenone (Sigma-Aldrich Rus LLC, Russia). The same ketones were used as reference components in determining indices of retention:



Approximately 100 mg of hydroxylamine sulfate (0.80 mM) and 40 mg sodium hydroxide (1 mM) were added to around 100 μL (or 100 mg for solids) of aldehyde (0.65–0.80 mM) 2–4 mL of isopropyl alcohol (reagent grade; Kriokhrom, St. Petersburg). The reaction mixtures were kept at room temperature for 24 h with periodic stirring and then diluted 10^3 times with the eluent to dose them into the chromatograph. Methanol (reagent grade; Kriokhrom, St. Petersburg) and deionized water (resistivity, 18.2 $\text{M}\Omega\text{ cm}$) were used to prepare eluents for HPLC. Aqueous acetonitrile solutions were degassed via filtration under vacuum and ultrasonic treatment using a 420 W Sapphire TTTs unit (Sapfir, Russia). The pH values of the eluent were approximately 6.2.

Conditions for Chromatographic Analysis

All oximes were characterized without isolating them from reaction mixtures, since they formed through a derivatization reaction [20]. Retention times were determined on a Shimadzu LC-20 Prominence liquid chromatograph with a diode matrix detector and a Phenomenex C18 column 250 mm long with an internal diameter of 4.6 mm (size of sorbent particles, 5 μm) in different isocratic modes. The eluent flow rate was 1.0 mL/min, and the temperature of the column was 30°C. The content of methanol in the eluent varied from 30 to 80 vol %. Samples were dispensed using a SIL-20A/AC autosampler (sample volume, 20 μL). The multiplicity of parallel determinations was 2–3, and the standard deviations of retention times of analytes in the range of 10 min was usually no more than 0.01–0.02 min.

Processing Results

Oximes were characterized according to logarithmic indices of retention under conditions of isocratic elution [1]:

$$RI_x = RI_n + (RI_{n+k} - RI_n) \times [\log(t_{R,x}) - \log(t_{R,n})] / [\log(t_{R,n+k}) - \log(t_{R,n})], \quad (1)$$

where $t_{R,x}$, $t_{R,n}$, and $t_{R,n+k}$ are the retention times of the target analyte (x) and the reference components closest to it in terms of parameters of retention with the values of indices of retention RI_n and RI_{n+k} assigned to them. n -Alkylphenyl ketones $C_6H_5COC_nH_{2n+1}$, $RI_n = 100n_C$, where n_C is the total number of carbon atoms in the molecule, were used as reference components in RP HPLC.

The retention times of theoretically unsorbed components (t_0) needed to calculate logarithmic indices of retention were estimated from the retention times of three reference n -alkylphenyl ketones using the Peterson and Hirsch formula [21]

$$t_0 \approx (t_{R,1}t_{R,3} - t_{R,2}^2) / (t_{R,1} + t_{R,3} - 2t_{R,2}). \quad (2)$$

Excel software (Microsoft Office, 2010) was used for statistical processing of the parameters of retention. The parameters of recurrent dependences were calculated with a step of $\Delta C = 10\%$ for varying concentrations of the organic solvent in eluents, and graphs were plotted using the Origin software (versions 4.1 and 8.1).

RESULTS AND DISCUSSION

Characterizing Indices of Retention

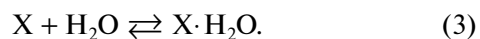
Oximes of nine aromatic aldehydes (IIa–i) and three aromatic ketones (IIj–l) were characterized in this work. Since one of our main goals was to consider indices of retention, Table 1 with RI values is presented first.

Table 1 shows substituents in the aryl fragments of molecules, the indices of retention (RI) of oximes and their precursors (aldehydes) when using eluents that differ by concentrations (C) of methanol from 30 to 80 vol % in steps of 10%, alongside relative optical densities $A_{rel} = A(254)/A(220)$ [5, 22, 23]. Considerable differences between A_{rel} values are evidence of the conversion of aromatic carbonyl compounds into corresponding oximes as a result of treatment with hydroxylamine. The periods (and thus indices) of retention of these compounds are in some cases close, but differing values of A_{rel} are a clear sign of a reaction occurring. The $RI(C)$ values were used to calculate coefficients dRI/dC of the dependence of RI on the content of methanol in the eluent and the corresponding coefficients of linear regression correlation (R). Additional comments on the identified anomalies are provided in the last column. The RI values of three aldehydes agree with ones determined independently

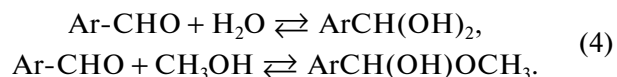
in [5], and the combined data sets were used to calculate dRI/dC . The indices of retention of alkyl aromatic ketones acetophenone, propiophenone, and butyrophenone are by definition 800, 900, and 1000, and are therefore not presented in Table 1.

A comparison of several $RI(C)$ values determined for eluents with $30 \leq C \leq 80$ vol % in addition to several A_{rel} values allows us to identify ones that are anomalous (presented in bold in Table 1). If they are greater than the average values for other concentrations of methanol, or if the corresponding points are located above the lines of regression, they are marked with \uparrow . If they are less, they are marked with \downarrow . However, the number of such anomalies identified when examining indices of retention is relatively small. For example, the RI values for 2-methylbenzaldehyde at $C = 30$ and 40% (805 and 806) are noticeably lower than the others (870–885). The same applies to the values of A_{rel} at $C = 30$ –40% (0.11–0.12) and others (4.3–4.8).

In reversed phase HPLC, anomalies in retention are often attributed to changes in the mechanisms of sorbate–sorbent interaction. However, if such anomalies are observed simultaneously for the parameters of retention and the A_{rel} values, they are clearly caused by a change in the chemical nature of the sorbates, and not in the mechanisms of sorption. Variations in A_{rel} mean that chemical changes affect chromophores in the molecules. At high contents of water in the eluent, one reason for variations in A_{rel} could be the reversible formation of hydrate forms of sorbates, which is consistent with the literature data known for many compounds [24–26]:



At the same time, we cannot exclude the reversible methanolysis of aldehydes, which is equivalent to the formation of covalent hydrates or hemiacetals:



Features of the A_{rel} values were noted for both 2-methylbenzaldehyde itself and its oxime at $C = 30\%$ (0.07, compared to 0.96–1.23). Similar anomalies in the values of RI and A_{rel} can be identified for 4-methylbenzaldehyde oxime at methanol concentrations of 30 and 40% in the eluent. There are examples of A_{rel} values at low concentrations of methanol that are overestimated relative to others: 4-hydroxybenzaldehyde, vanillin and 3,4-dimethoxybenzaldehyde. However, the reproducibility of spectral ratios $A_{rel} = A(254)/A(220)$ is generally low and depends on the absolute intensity of chromatographic peaks that could be due to impurities absorbing in the UV region in the eluent.

Finally, unexpected examples of chromatographic behavior were revealed when comparing the indices of retention of 2- and 4-hydroxybenzaldehydes and main

Table 1. Indices of retention of aromatic carbonyl compounds and their oximes at different concentrations of methanol in the eluent (RI), relative optical densities $A_{rel} = A(254/220)$, dRI/dC values and differences in oxime–aldehyde indices of retention (ΔRI). Anomalous values are highlighted in bold, indicating the direction of deviation (\uparrow or \downarrow). A dash means that RI and A_{rel} were not determined at this concentration of methanol

Substituents in the phenyl fragment	Index of retention, A_{rel} and ΔRI	Concentration of methanol in the eluent, C, vol %						Average values A_{rel}	dRI/dC (R), comments
		30	40	50	60	70	80		
2-Methyl (a)	RI (aldehyde)			870	878	885	876		
	A_{rel}			4.60	4.77	4.79	4.27	4.6 ± 0.2	
	RI (aldehyde) [5]	–	–	870	874	879	884		0.36 ± 0.13 (0.76)*
	A_{rel} [5]	–	–	2.51	2.00	1.71	1.52	1.9 ± 0.4	
	RI (oxime)	796	826	846	837	825	799		–1.5 ± 0.3 (–0.968)
	A_{rel}		0.93	1.18	1.23	0.96	1.17	1.09 ± 0.14	
4-Methyl (b)	ΔRI	–	–	–24; –14	–41; –23	–60; –35	–77; –62		
	RI (aldehyde)	–	–	857	857	858	846		
	A_{rel}	–	–	3.63	3.93	3.39	3.20	3.5 ± 0.3	
	RI (aldehyde) [5]	–	–	860	860	860	861		–0.14 ± 0.15 (–0.35)*
	A_{rel} [5]	–	–	2.36	2.01	2.02	1.97	2.1 ± 0.2	
	RI (oxime)	–	806↓	855	855	842	813		–1.4 ± 0.5 (–0.906)
2-Hydroxy (c)	A_{rel}	–	0.83↓	1.82	1.78	1.07	1.86	1.6 ± 0.4	Value of $A(40)$ is lower than others
	ΔRI	–	–	–2; –5	–2; –5	–16; –18	–33; –48		
	RI (aldehyde)	782	785	790	798	802	798		
	A_{rel}	0.92	0.91	0.84	0.61	0.60	0.59	0.74 ± 0.16	
	RI (aldehyde) [5]	–	–	795	804	811	819		0.58 ± 0.13 (0.85)*
	RI (oxime)	780	785	792	799	808	805		
	A_{rel}	0.88	0.86	0.80	0.72	0.82	0.77	0.80 ± 0.06	Values of RI and A practically coincide with the data for aldehyde
	ΔRI	–2	0	+2; –3	+1; –5	+6; –3	+7; –12		

Table 1. (Contd.)

Substituents in the phenyl fragment	Index of retention, A_{rel} and ΔRI	Concentration of methanol in the eluent, C, vol %							Average values A_{rel}	dRI/dC (R), comments
		30	40	50	60	70	80	80		
4-Hydroxy (d)	RI (aldehyde)	706	694	751	796	846	886		4.6 ± 0.1 (0.9992)	
	A_{rel}			0.04	0.04	0.04	0.04		0.04 ± 0.00	
	RI (oxime)	747	798	792	800	846	886		3.3 ± 0.6** (0.969)	
	A_{rel}			0.02	0.07	0.07	0.04		0.04 ± 0.02	
	ΔRI	–	–	41	4	0	0			Values of RI and A virtually coincide with the data for aldehyde
2-Methoxy (e)	RI (aldehyde)	–	–	902	907	908	892		–0.29 ± 0.34 (–0.51)	
	A_{rel}	–	–	0.08	0.08	0.08	0.05		0.07 ± 0.02	
	RI (oxime)	822	805	799	788	772	746		–1.4 ± 0.1 (–0.978)	
	A_{rel}	0.75	0.71	0.76	0.67	0.76	0.54		0.70 ± 0.08	
	ΔRI			–103	–119	–136	–146			
4-Methoxy (f)	RI (aldehyde)	802	793	790	789	789	782		–0.32 ± 0.07 (–0.92)	
	A_{rel}	0.52	0.49	0.52	0.49	0.54	0.63		0.53 ± 0.05	
	RI (oxime)	794	778	765	753	741	708		–1.31 ± 0.04 (–0.998)	
	A_{rel}	1.23	1.24	1.34	1.29	1.28	0.52↓		1.28 ± 0.04	
	ΔRI	–8	–15	–25	–36	–48	–74			
4-Hydroxy-3-methoxy (Vanillin, g)	RI (aldehyde)	699	707	749	797	846	887		4.6 ± 0.1 (0.9995)	
	A_{rel}	0.33↑	0.04	0.05	0.04	0.04	0.04		0.04 ± 0.01	
	RI (oxime)	672	655	643	635	623	611		–1.17 ± 0.06 (–0.995)	
	A_{rel}	0.52	0.54	0.54	0.53	0.55	0.54		0.54 ± 0.01	
	ΔRI	–27	–52	–106	–162	–223	–276			
3-Hydroxy-4-methoxy (Isovanillin, h)	RI (aldehyde)	746	706	750	796	846	886		4.6 ± 0.1 (0.9995)	
	A_{rel}	0.05	0.04	0.05	0.05	0.05	0.04		0.05 ± 0.01	
	RI (oxime)	691	669	653	642	630	614		–1.47 ± 0.09 (–0.993)	
	A_{rel}	0.54	0.55	0.53	0.53	0.53	0.52		0.53 ± 0.01	
	ΔRI	–55	–47	–97	–154	–216	–272			

Table 1. (Contd.)

Substituents in the phenyl fragment	Index of retention, A_{rel} and ΔRI	Concentration of methanol in the eluent, C, vol %						Average values A_{rel}	dRI/dC (R), comments
		30	40	50	60	70	80		
3,4-Dimethoxy (i)	RI (aldehyde)	760	739	750	797	845	887		4.6 ± 0.1 (0.9996)
	A_{rel}			0.04	0.04	0.04	0.04	0.04 ± 0.00	
	RI (oxime)	753	724	707	694	680	668		-1.63 ± 0.14 (-0.985)
	A_{rel}	0.58	0.34	0.59	0.62	0.60	0.57	0.55 ± 0.10	
	ΔRI	-7	-15	-43	-103	-165	-219		
Acetophenone (j)	A_{rel} (ketone)	-	-	3.34	2.85	2.78	2.53	2.8 ± 0.3	
	RI (oxime)	816	802	803	803	810	800		
	A_{rel}	3.70	3.51	3.31	2.12	2.21	1.97	2.8 ± 0.3	**
	ΔRI	16	2	3	3	10	0		Values of RI and A virtually coincide with the data for aldehyde
	A_{rel} (ketone)	-	-	1.98	2.56	2.29	1.82	2.2 ± 0.3	
Propiophenone (k)	RI (oxime)	887	872	915	902	893	862		-1.7 ± 0.3 (-0.962)
	A_{rel}	0.34↓	0.40↓	1.14	1.16	1.18	0.99	1.12 ± 0.09	Values of A(30) and A(40) are lower than others
	ΔRI	-13	-28	15	2	-7	-38		
	A_{rel} (ketone)	-	-	2.78	2.76	2.52	2.37	2.6 ± 0.2	
	RI (oxime)	-	968	1015	1015	987	948		-2.3 ± 0.6 (-0.932)
Butyrophenone (l)	A_{rel}		0.29↓	1.09	1.12	1.14	1.13	1.12 ± 0.02	
	ΔRI		-32↓	15	15	-13	-52		Value of A(40) is lower than neighboring value A(50)

* Values of dRI/dC (R), calculated from combined sets of RI values in this work and [5], are given for the indicated aldehydes.** The value of dRI/dC is excluded from consideration due to the hydrolysis of this oxime during chromatographic separation. (See comments in text.)

Table 2. Values of hydrophobicity factors $\log P$

Carbonyl compound	$\log P$	$\log P$ of oxime
Benzaldehyde	1.46 ± 0.02	1.49
2-Hydroxybenzaldehyde	1.83 ± 0.19	1.88
4-Hydroxybenzaldehyde	1.3	1.2
4-Methoxybenzaldehyde	1.7	1.5
Acetophenone	1.70 ± 0.09	1.88
Propiophenone	2.23 ± 0.05	2.27

components of their reaction mixtures with hydroxylamine. The RI values of 2-hydroxybenzaldehyde and the corresponding reaction product differed by only (-2) – $(+7)$ units at all concentrations of methanol in the eluent, and A_{rel} were 0.74 ± 0.16 and 0.80 ± 0.06 , respectively (i.e., they virtually coincided with one another). When the content of methanol in the eluent was $C = 50\%$, the index of retention of 4-hydroxybenzaldehyde differed statistically from the RI of the reaction mixture component (751 and 792, respectively) and the retention times (8.80 and 10.82 min; see below). However, they were almost identical when C was raised to 60–80%. If we compare this to the equality of the A_{rel} values (Table 1), we must conclude they belong to the same compounds (aldehydes). Such similarity between the values of RI and A_{rel} was noted for acetophenone and the main component of its reaction mixture with hydroxylamine. No such matches were identified for homologs propiophenone and butyphenone.

There could be several reasons for this. Theoretically, acetophenone and 2- and 4-hydroxybenzaldehydes might not react with hydroxylamine under the chosen conditions. However, this seems unlikely because such anomalies are not observed for other aromatic aldehydes (including 4-hydroxy-3-methoxy- and 3-hydroxy-4-methoxybenzaldehydes) and other ketones. Another possibility is the rapid hydrolysis of oximes when the reaction mixtures are dissolved in large amounts of water-containing eluent at the stage of sample preparation. The hydrolysis of oximes during separation is also possible as their chromatographic zones move along the column. This distorts the shapes of chromatographic peaks and was observed in [5] for unsubstituted hydrazones of aromatic aldehydes. The second of the reasons given above would therefore seem to be the one most likely. If so, the values of the $d\text{RI}/dC$ coefficients calculated for such problematic oximes are incorrect and should be excluded from further consideration.

The conclusion drawn in [7] about oximes being more resistant to hydrolysis than unsubstituted hydrazones is most likely true only for individual representatives of those classes. Considering the stability noted in [5] for unsubstituted hydrazones of aromatic ketones in RP HPLC, relative to that of hydrazones of

aromatic aldehydes, we may conclude there are examples of different behavior in both classes of compounds under such conditions.

The series of characterized oximes (IIa–i) includes substituents that differ in nature (methyl, hydroxy and methoxy groups), and thus chromophores (derivatives of the simplest aromatic aldehydes). It is therefore not surprising that their characteristics (including their $d\text{RI}/dC$ values) differ markedly. It was concluded in [27] that the more polar (hydrophilic) the sorbate, the lower coefficient $d\text{RI}/dC$. However, the different polarity characteristics (according to [28], there are more than one hundred of them) of aromatic carbonyl compounds and their corresponding oximes do not allow us to choose the most polar ones. Judging from the active hydrogen atom in the molecules, we may assume that oximes are more hydrophilic. However, if we compare the classical polarity characteristics of organic compounds (dipole moments (μ) and dielectric permeabilities (ϵ)), they are stronger for carbonyl compounds. For example, $\mu = 2.9$ D and $\epsilon = 17.8$ for benzaldehyde, while $\mu = 0.9$ D and $\epsilon = 3.8$ for benzaldoxime. On the other hand, the values of hydrophobicity factors $\log P$, known only for a limited number of structural analogs, show that aromatic carbonyl compounds and their oximes are comparable with regard to this parameter (Table 2).

At the same time, the ratio of $d\text{RI}/dC$ values, which can be considered another characteristic of polarity, is the opposite for aromatic carbonyl compounds and their oximes. If the $d\text{RI}/dC$ values vary in the range of (-0.3) – 4.6 for the aldehydes themselves, they are smaller and belong to a narrower range (-1.2) – (-1.6) for the corresponding oximes (Table 1). This pattern explains an important chromatographic feature of aromatic aldehyde oximes in RP HPLC. If $d\text{RI}/dC > 0$ for aldehydes and $d\text{RI}/dC < 0$ for their oximes, the differences between indices of retention $\Delta\text{RI} = \text{RI}(\text{oxime}) - \text{RI}(\text{aldehyde})$ are not constant but diminish to varying degrees as the concentration of in the eluent rises. This is confirmed by the data in Table 1 for all substituted benzaldehydes: $(-19) \rightarrow (-70)$ (a), $(-8) \rightarrow (-42)$ (b), $(-103) \rightarrow (-146)$ (e), $(-8) \rightarrow (58)$ (f), $(-52) \rightarrow (-276)$ (g), $(-55) \rightarrow (-272)$ (h), and $(-43) \rightarrow (-219)$ (i). Such examples greatly complicate the use of addi-

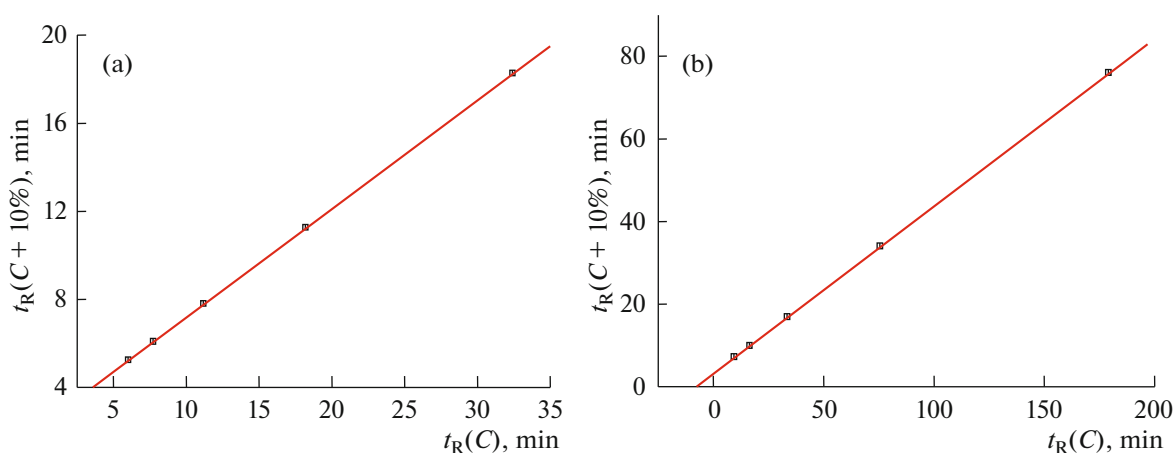


Fig. 1. Recurrent approximation of the retention times of (a) acetophenone and (b) butyrophenone, illustrating the absence of anomalies in their retention times. Parameters of linear regression equations: (a) $a = 0.4932 \pm 0.0005$, $b = 2.231 \pm 0.008$, $R = 1.000$, $S_0 = 0.01$; (b) $a = 0.4059 \pm 0.0003$, $b = 3.05 \pm 0.02$, $R = 1.000$, $S_0 = 0.04$.

tive schemes for assessing indices of retention in RP HPLC, and in some cases they make it impossible.

Recurrent Approximation of the Retention Times of Aromatic Carbonyl Compounds and Their Oximes

Determining and characterizing indices of retention for sorbates in RP HPLC does not prevent independent, parallel consideration of their retention times. If the aim of such consideration is to identify anomalies in chromatographic retention, the most informative way of presenting data is the recurrent approximation of retention times [29]:

$$t_R(C + \Delta C) = at_R(C) + b, \quad (5)$$

where $\Delta C = \text{const}$ is a given constant step of variation in the concentration of the organic component of the eluent, and coefficients a and b are calculated according to least squares.

We are thus talking about recurrent control of the retention times of the characterized sorbates. Retention times of the characterized carbonyl compounds and their oximes in the 30 to 80% range of methanol concentrations in the eluent are given in Table 3. It additionally duplicates the values of A_{rel} , which seems appropriate for comparing them to retention times. The main feature of the data is that the recurrent representation of retention times allows us to identify many more of their anomalies than considering indices of retention. Since recurrent approximations of $t_R(C)$ dependences of the form (3) are linear in ideal cases, anomalous t_R values can be identified according to deviations of points from lines of regression. Anomalous values are highlighted in bold, indicating the nature of the deviations: overestimated (\uparrow) or underestimated (\downarrow), relative to others.

When commenting on the data in Table 3, it should be noted that acid–base equilibria of the form $B + H^+ \rightleftharpoons [BH]^+$, which are one reason for the recurrent approximation of retention times deviating from linearity [29], can be ignored when it comes to oximes. The pK_a values of oximes actually exceed the pH of the eluent (approx. 6.2):

Oxime	pK_a
Benzaldehyde	11.3 ± 0.1
2-Hydroxybenzaldehyde	9.1 ± 0.1
2-Methoxybenzaldehyde	11.9
Acetophenone	11.4 ± 0.1

The lower pK_a value of 2-hydroxybenzaldehyde is due to it containing a phenolic hydroxyl group.

The initial premise for identifying factors that influence retention times of sorbates is that recurrent dependencies (4) are linear when there are no anomalies (coefficients of correlation exceed 0.999). This was noted for several retention times in [29], but it seems appropriate to consider it further in light of its particular importance. Examples of such compounds are *n*-alkyl phenyl ketones (reference components for determining indices of retention in RP HPLC). Figure 1 shows the corresponding dependences for (a) acetophenone and (b) butyrophenone.

Similar linear dependences characterize the behavior of the oximes of 4-methoxy-, 4-hydroxy-3-methoxy-, and 3-hydroxy-4-methoxybenzaldehyde. Recurrent approximations of the retention of remaining oximes are characterized by certain anomalies. Points $t_R(30)$ – $t_R(40)$ corresponding to the highest content of water in the eluent thus deviate downward from the regression lines in the graphs of dependence (4) for the retention times of oximes of 2-methoxy-

Table 3. Results from recurrent monitoring of retention times of aromatic carbonyl compounds and their oximes in combination with relative optical densities $A_{rel} = A(254/220)$. Anomalous values are highlighted in bold, indicating the direction of their deviation. A dash means that R_I and A_{rel} were not determined at this concentration of methanol

Substituents in the phenyl fragment	Retention time and A_{rel}	Concentration of methanol in the eluent, C, vol %						R	Comments
		30	40	50	60	70	80		
2-Methyl (a)	t_R (aldehyde)	33.817	19.023↑	16.248	10.316	7.354	5.839	0.99999	Ejection of point $t_R(40) - t_R(50)$
	A_{rel}			4.60	4.77	4.79	4.27		
	t_R (oxime)	31.262	21.736	14.261	8.869	6.399↑	5.220↑	0.9997	Points $t_R(70) - t_R(80)$ and $t_R(60) - t_R(70)$ deviate upward from the regression line
	A_{rel}		0.93	1.18	1.23	0.96	1.17		
4-Methyl (b)	t_R (aldehyde)	33.373	17.484↑	15.124	9.536	6.896	5.587	0.9998	Ejection of point $t_R(40) - t_R(50)$
	A_{rel}	0.48↓	0.50↓	3.63	3.93	3.39	3.20		Values of $A(30)$ and $A(40)$ are lower than others
	t_R (oxime)	–	18.953↑	15.001	9.459	6.636	5.320	0.9998	Point $t_R(40) - t_R(50)$ deviates upward from the regression line
	A_{rel}	–	0.83↓	1.82	1.78	1.07	1.86		Value of $A(40)$ is lower than others
2-Hydroxy (c)	t_R (aldehyde)	27.852	16.431	10.702	7.713	6.074	5.211	0.9998	No anomalies
	A_{rel}	0.92	0.91	0.84	0.61	0.60	0.59		No anomalies
	t_R (oxime)	27.586	16.432	10.798	7.738	6.166	5.266		Values of t_R and A correspond to the original aldehyde (see comments in the text)
	A_{rel}	0.88	0.86	0.80	0.72	0.82	0.77	0.9996	Points $t_R(30) - t_R(40)$ and $t_R(40) - t_R(50)$ deviate in different directions from the regression line
4-Hydroxy (d)	t_R (aldehyde)	14.982↓	9.158↑	8.800	7.684	6.709	5.930		
	A_{rel}			0.04	0.04	0.04	0.04		
	t_R (oxime)	21.852↑	18.913	10.817	7.772	6.699	5.981	1.000	Point $t_R(30) - t_R(40)$ deviates upward from the regression line
	A_{rel}			0.02	0.07	0.07	0.04		

Table 3. (Contd.)

Substituents in the phenyl fragment	Retention time and A_{rel}	Concentration of methanol in the eluent, C, vol %						R	Comments
		30	40	50	60	70	80		
2-Methoxy (e)	t_R (aldehyde)	–	–	19.337	11.556	7.772	5.978	0.99998	No anomalies
	A_{rel}	–	–	0.08	0.08	0.08	0.05		No anomalies
	t_R (oxime)	39.031↓	18.916	11.151	7.460	5.700	4.853	1.000	Point $t_R(30)-t_R(40)$ deviates downward from the regression line
	A_{rel}	0.75	0.71	0.76	0.67	0.76	0.54		No anomalies
4-Methoxy (f)	t_R (aldehyde)	33.097	17.458	10.678	7.495	5.905	5.093	0.9996	No anomalies
	A_{rel}	0.52	0.49	0.52	0.49	0.54	0.63		No anomalies
	t_R (oxime)	30.999	15.673	9.448	6.647	5.340	4.610	0.9993	No anomalies
	A_{rel}	1.23	1.24	1.34	1.29	1.28	0.52↓		Value of $A(80)$ is lower than others
4-Hydroxy-3-methoxy (Vanillin, g)	t_R (aldehyde)	14.167↓	9.895	8.720	7.689	6.705	5.930	0.9993	Point $t_R(30)-t_R(40)$ deviates downward from the regression line
	A_{rel}	0.33↑	0.04	0.05	0.04	0.04	0.04		Value of $A(30)$ is greater than others
	t_R (oxime)	11.444	7.250	5.476	4.698	4.286	4.077	0.9994	No anomalies
	A_{rel}	0.52	0.54	0.54	0.53	0.55	0.54		No anomalies
3-Hydroxy-4-methoxy (Isovanillin, h)	t_R (aldehyde)	20.820↓	9.862	8.782	7.676	6.702	5.920	0.9993	Point $t_R(30)-t_R(40)$ deviates downward from the regression line
	A_{rel}	0.05	0.04	0.05	0.05	0.05	0.04		No anomalies
	t_R (oxime)	13.344	7.884	5.700	4.786	4.332	4.094	0.9993	No anomalies
	A_{rel}	0.54	0.55	0.53	0.53	0.53	0.52		No anomalies
3,4-Dimethoxy (i)	t_R (aldehyde)	23.251↓	12.153↓	8.796	7.683	6.692	5.932	0.998	Points $t_R(30)-t_R(40)$ and $t_R(40)-t_R(50)$ deviate downward from the regression line
	A_{rel}	0.31↑	0.34↑	0.04	0.04	0.04	0.04		Values of $A(30)$ and $A(40)$ are greater than others
	t_R (oxime)	21.929↓	11.074	7.174	5.534	4.747	4.374	0.9995	Point $t_R(30)-t_R(40)$ deviates downward from the regression line
	A_{rel}	0.58	0.34	0.59	0.62	0.60	0.57		No anomalies

Table 3. (Contd.)

Substituents in the phenyl fragment	Retention time and A_{rel}	Concentration of methanol in the eluent, C, vol %						R	Comments
		30	40	50	60	70	80		
Acetophenone (j)	t_R (ketone)	32.46	18.24	11.23	7.774	6.052	5.228	1.000	No anomalies
	A_{rel}	—	—	3.34	2.85	2.78	2.53	2.8 ± 0.3	No anomalies
	t_R (oxime)	37.194↓	18.494	11.428	7.852	6.176	5.264	0.9998	The t_R and A values are close to the values for the original ketone. Point $t_R(30)-t_R(40)$ deviates downward from the regression line
	A_{rel}	3.70	3.51	3.31	2.12	2.21	1.97		
Propiophenone (k)	t_R (ketone)	75.82	36.76	19.15	11.232	7.624	6.950	1.000	No anomalies
	A_{rel}	—	—	1.98	2.56	2.29	1.82	2.2 ± 0.3	No anomalies
	t_R (oxime)	67.65↓	30.02↑	20.86	11.306	7.50	5.719	0.9993	Points $t_R(30)-t_R(40)$ and $t_R(40)-t_R(50)$ deviate in different directions from the regression line
	A_{rel}	0.34↓	0.40↓	1.14	1.16	1.18	0.99		Values of $A(30)$ and $A(40)$ are lower than others
Butyrophenone (l)	t_R (ketone)	179.52	75.91	33.86	16.80	9.82	7.07	1.000	No anomalies
	A_{rel}	—	—	2.78	2.76	2.52	2.37	2.6 ± 0.2	No anomalies
	t_R (oxime)	—	60.15↑	36.86	17.89	9.49	6.515	0.998	Point $t_R(40)-t_R(50)$ deviates upward from the regression line
	A_{rel}	—	0.29↓	1.09	1.12	1.14	1.13		Value of $A(40)$ is lower than others

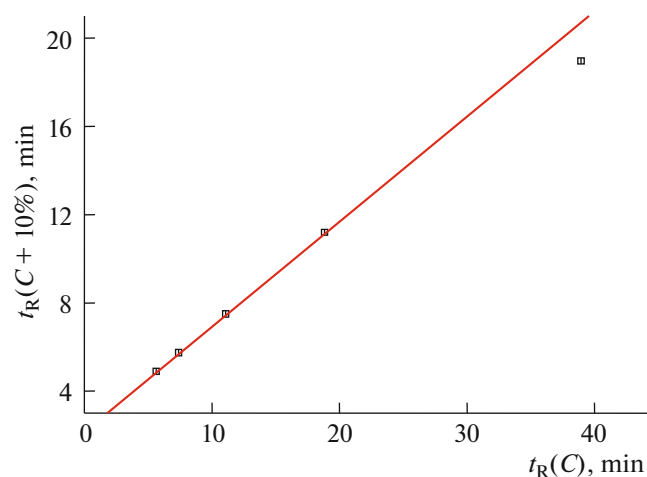


Fig. 2. Illustration of the anomaly of recurrent approximation of retention times using the example of 2-methoxybenzaldehyde oxime. Parameters of linear regression equations (without allowing for the anomalous point) are $a = 0.4763 \pm 0.0006$, $b = 2.144 \pm 0.007$, $R = 1.000$, $S_0 = 0.006$.

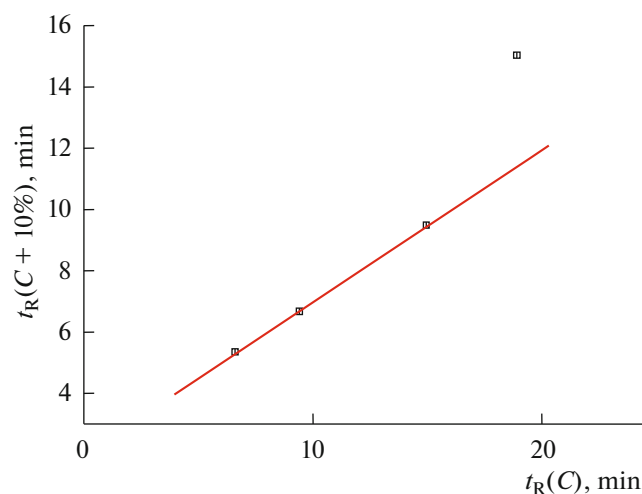


Fig. 3. Illustration of the anomaly of recurrent approximation of retention times using the example of 4-methylbenzaldehyde oxime. Parameters of linear regression equations (without allowing for the anomalous point) are $a = 0.50 \pm 0.01$, $b = 1.99 \pm 0.11$, $R = 0.9998$, $S_0 = 0.06$.

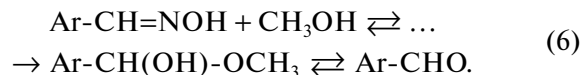
and 3,4-dimethoxybenzaldehydes. This type of recurrent dependences indicates reversible hydration of sorbates (Eq. (3)) [29, 30]. Experimental evidence of hydrate formation was obtained for 4-hydroxybenzaldehyde oxime in [31, 32].

For a graphic illustration of such an anomaly, we can choose, e.g., data for 2-methoxybenzaldehyde oxime (Fig. 2). Similar deviations were also recorded for oximes of aceto- and propiophenones (Table 3). It is interesting that such anomalies were observed earlier only when using eluents containing acetonitrile instead of methanol [29]. This is because methanol forms hydrates whose stability is greater than that of many organic compounds.

Finally, previously unobserved anomalies in the recurrent approximation of retention times were recorded for oximes 4-methylbenzaldehyde and 4-hydroxybenzaldehyde. Points $t_R(30) - t_R(40)$, which correspond to the highest content of water in the eluent, deviate not downward but upward from the regression lines. Figure 3 illustrates such an anomaly using the example of 4-methylbenzaldehyde oxime.

If the downward deviation of the points at the highest content of water in the eluent is consistent with the formation of more hydrophilic hydrate forms of sorbates [29], upward deviation of the points should indicate the changing of sorbates into more hydrophobic forms, according to the same logic. This issue certainly deserves more detailed consideration, so we may now limit ourselves to assuming that the reason for such t_R anomalies might not be hydration but the partial hydrolysis of oximes with the formation of the corresponding aldehydes. If such hydrolysis occurs as the chromatographic zones of the sorbates move along

the column, it may not result in products of hydrolysis registering in the form of separate peaks. An alternative process could be the methanolysis of oximes with the formation of hemiacetals or ketals, which cannot be preliminarily isolated from solutions due to their instability, but are more hydrophobic than either oxime or the corresponding carbonyl compounds:



If we return to considering anomalies in the recurrent approximation of the retention times of the initial aldehydes, there are examples of where there are none (2-hydroxy-, 2-methoxy-, and 4-methoxybenzaldehydes, and all alkylphenyl ketones, as was noted above) and points deviate downward due to hydration (4-hydroxy-3-methoxy-, 3-hydroxy-4-methoxy-, and 3,4-dimethoxybenzaldehydes). Along with this, more complex cases of deviations that might require more detailed consideration have been noted for 2-methyl-, 4-methyl-, and 4-hydroxybenzaldehydes.

We may assume the reason for such cases could be the hydration or methanolysis of aldehydes (4). Figure 4 illustrates this using 4-hydroxybenzaldehyde as an example.

This assumption is also supported by anomalies in the relative optical densities of A_{rel} : at 30 and 40% concentrations of methanol in the eluent, since they differ considerably from other A_{rel} values. For example, the values of RI(30) and RI(40) are much lower for 2-methylbenzaldehyde than the others, as are the values of A(30) and A(40). The drop in A_{rel} is easily explained by the destruction of the Ar-CO chromo-

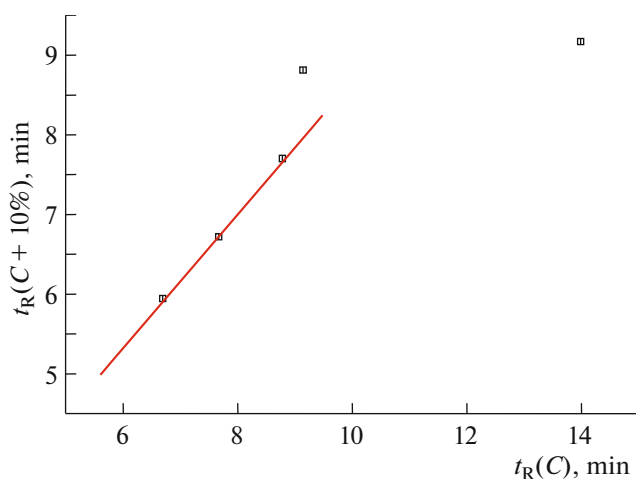


Fig. 4. Illustration of anomalies in the recurrent approximation of retention times using the example of 4-hydroxybenzaldehyde. Parameters of linear regression equations (without allowing for two anomalous points) are $a = 0.84 \pm 0.02$, $b = 0.27 \pm 0.20$, $R = 0.9996$, $S_0 = 0.04$.

phore of aromatic carbonyl compounds during processes (4) and (6).

CONCLUSIONS

The example of oximes of aromatic carbonyl compounds was used to show that detailed characterization of chromatographic properties of sorbates in reversed phase HPLC must include determining and recurrent monitoring of their retention times (more precisely, the dependence of retention times on the concentrations of the organic component of the eluent). Such monitoring allows us to identify considerably more anomalies in the retention times than other means. The cause of most observed anomalies is not variations in their mechanisms of retention, but changes in the chemical nature of the sorbates due to their interaction with components of the eluent. To confirm this, chromatographic data must be supplemented with spectral parameters, specifically relative optical densities $A_{\text{rel}} = A(\lambda_2)/A(\lambda_1)$. Variations in A_{rel} values indicate a change in the nature of the chromophores in the molecules and thus chemical transformations of the sorbates.

Joint examination of the indicated characteristics of oximes allowed us to discover among them compounds that are stable under conditions of RP HPLC separation and identify examples of their reversible hydration (oximes of 2-methoxy- and 3,4-dimethoxybenzaldehydes) and irreversible hydrolysis (oximes of 2- and 4-hydroxybenzaldehydes, acetophenone oxime) to form the corresponding aldehydes. It was established that coefficients of the dependence of the indices of retention on concentration dRI/dC of the organic component of the eluent for aldehydes are

most often greater than zero, while $dRI/dC < 0$ for their oximes. The differences between indices of retention $\Delta RI = RI(\text{oxime}) - RI(\text{aldehyde})$ are consequently not constant. Instead, they fall as the concentration of methanol in the eluent rises, which limits the applicability of additive schemes for assessing indices of retention in RP HPLC.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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