Retention indices of some β-adrenolytics and their perfluoroacyl derivatives

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Retention indices (RI) of 10 β-adrenolytics and their trifluoroacetyl and heptafluorobutyryl derivatives on columns with nonpolar OV 1 and moderately polar OV 17 stationary phases, respectively, have been studied. The retention indices of the original drugs on the OV 1 phase ranged from 1721 to 2795 and on the OV 17 phase from 1939 to 3290. By derivatization the differences in RI values in the systems with different stationary phases decreased and so did the differences in values obtained in different laboratories.

Исследованы индексы удерживания (RI) десяти β-адренолитических препаратов и их трифторацетил- и гептафторбутирил-производных на колоннах с неполярным носителем OV 1 и умеренно полярным носителем OV 17. Индексы удерживания исходных препаратов на носителе OV 1 находились в промежутке от 1721 до 2795, а на носителе OV 17 между 1939 и 3290. В результате дериватизации различия в значениях RI систем с различными носителями уменьшились. Уменьшились также различия в величинах, определенных в разных лабораториях.

Retention indices (RI), introduced into gas chromatography by *Kovats* in 1958 [1], belong to the most frequently used characteristics describing the motion of organic compounds in a chromatographic system. In the literature concerned with analysis of drugs the relationships of RI with temperature of measurement [2], type of the biological matrix, method of isolation, and the amount of the analyzed drug [3] were discussed and the differences in RI values on packed and capillary columns [4] were investigated.

β-Adrenolytics belong to the group of cardiovascularly active drugs. Their main representative, propranolol, is, at present, one of the most frequently prescribed drugs at all [5]. The earliest works dealing with gas chromatographic determination of these drugs were published at the end of the sixties. Since then, tens of procedures for GLC-determination of β-adrenolytics have appeared in the literature. One of the most frequently used procedures includes derivatization of these drugs with anhydrides of perfluorated organic acids, mostly trifluoroacetic (TFAA) and heptafluorobutyric acids (HFBA), chromatograph-

ic separation of the obtained derivatives on OV 1 and OV 17 stationary phases, and subsequent detection with electron-capture detectors [6].

The aim of the present work was to complete the missing RI data of some β-adrenolytics and their most frequently used derivatives and to find out inter-laboratory differences in RI values of drugs belonging to this group.

Experimental

Ten β-adrenolytics have been studied. Five of them, *i.e.* metipranolol, nadolol, oxprenolol, propranolol, and toliprolol (all in the form of chlorides) were commercial products, four were gifts from the individual producers, namely, acebutolol (chloride) from May and Baker, Dagenham, Great Britain, alprenolol (chloride) and metoprolol (tartrate) from Hässle, Mölndal, Sweden, and bunolol (chloride) from Warner Lambert, Ann Arbor, U.S.A. The potential β-adrenolytic of Czechoslovak production, exaprolol (chloride), was from the Research Institute of Drugs, Modra, Czechoslovakia.

Derivatization both with TFAA and HFBA (Fluka, Buchs, Switzerland) was carried out according to the following procedure:

To aqueous solution of β-adrenolytic (1 cm^3, $\varrho = 100\,mg\,dm^{-3}$, calculated to free base) in 10-cm^3 test tubes with teflon cap 5 M-KOH solution (0.1 cm^3) and ethyl acetate (3 cm^3) were added. The solution was shaken in horizontal position for 10 min. After separation of the layers, the organic layer was transferred into a 3-cm^3 conical vial (Reacti-Vial, Pierce, Oud-Beijerland, The Netherlands) and evaporated under nitrogen flow at 50 °C. Then anhydride (0.1 cm^3) was added and derivatization proceeded at 50 °C for 30 min. The excess anhydride was evaporated and the residue was dissolved in benzene (0.1 cm^3).

In the case of determination of the original drugs benzene (0.1 cm^3) was added to the dry residue after extraction with ethyl acetate.

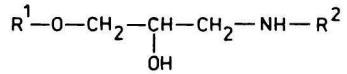
Gas chromatography was performed on a Perkin—Elmer 900 apparatus with flame ionization detector using columns (1 m long, 2 mm I.D.) packed with Chromosorb W AW DMCS (149—177 μm) coated with 5 % OV 1 and 2 % OV 17 stationary phases, respectively. Column temperature was 210 °C, injector temperature 220 °C, and detector temperature 280 °C. Nitrogen was used as a carrier gas at a flow rate of 40 $cm^3\,min^{-1}$. The amount of the injected sample was $10^{-3}\,cm^3$.

The retention indices were calculated according to the method published by *Grobler* and *Bálizs* [7]. n-Alkanes C_{20} to C_{30} were used as standards.

Results and discussion

Retention indices of 10 β-adrenolytics and their trifluoroacetyl (TFA) and heptafluorobutyryl (HFB) derivatives are listed in Table 1. In the case of acebutolol and nadolol the products of derivatization could not be analyzed because the reaction with anhydrides resulted in a mixture of products which were unstable under the conditions of analysis [8, 9]. The derivatives of all other β-adrenolytics were well analyzable.

Table 1. Retention indices of β-adrenolytics and their trifluoroacetyl (TFA) and heptafluorobutyryl (HFB) derivatives



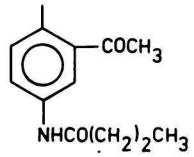
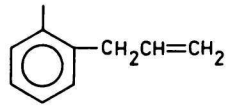
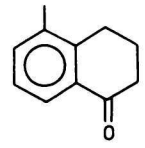
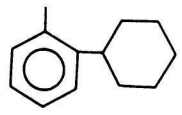
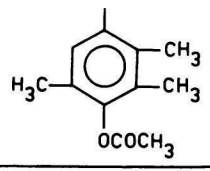
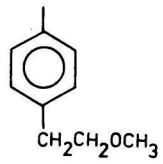
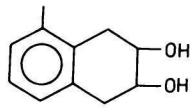
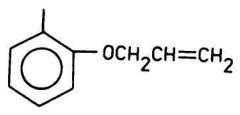
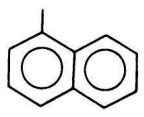
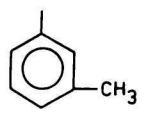
Drug	R^1	R^2	Retention indices					
			Original		TFA		HFB	
			OV 1	OV 17	OV 1	OV 17	OV 1	OV 17
Acebutolol		$CH(CH_3)_2$	2795	3290	—[a]	—[a]	—[a]	—[a]
Alprenolol		$CH(CH_3)_2$	1840	2044	1861	1975	1861	1892
Bunolol		$C(CH_3)_3$	2352	2644	2088	2431	2111	2390
Exaprolol		$CH(CH_3)_2$	2169	2379	2170	2293	2160	2179
Metipranolol		$CH(CH_3)_2$	1951	2173	1990	2073	2060	1984

Table 1 (Continued)

Drug	R^1	R^2	Retention indices					
			Original		TFA		HFB	
			OV 1	OV 17	OV 1	OV 17	OV 1	OV 17
Metoprolol		$CH(CH_3)_2$	2041	2294	2045	2202	2024	2107
Nadolol		$C(CH_3)_3$	2633	3031	— [a]	— [a]	— [a]	— [a]
Oxprenolol		$CH(CH_3)_2$	1927	2169	1930	2074	1928	1989
Propranolol		$CH(CH_3)_2$	2152	2417	2166	2309	2133	2209
Toliprolol		$CH(CH_3)_2$	1721	1939	1728	1858	1748	1769

a) Undeterminable derivatives.

From comparison of the RI values of β-adrenolytics and their TFA and HFB derivatives it is obvious that derivatization affected more significantly the RI values obtained on the more polar OV 17 phase. With the increasing size of the acyl residue the RI values decreased and in the case of HFB derivatives they were, except of bunolol, practically equal to the RI values obtained on the nonpolar OV 1 phase. These changes reflect broken polar interactions between the drug and the polar phase, brought about by delocalization of free electrons on oxygen and nitrogen by electron-accepting perfluoroacyl residues as well as by steric shielding of these electrons.

The effect of derivatization as well as of the size of the agent on RI values is relatively large in the case of the more polar OV 17 phase. The difference in RI of β-adrenolytics and their TFA derivatives ranged from 69 to 334 units and in the case of HFB derivatives from 152 to 478 units. This effect with the nonpolar OV 1 phase was in most cases not so significant. Bunolol and metipranolol were exceptions. It means that the increase of the relative molecular mass on binding two acyl residues by one molecule of β-adrenolytics (67—87 % in the case of di-TFA derivatives and 135—177 % in the case of di-HFB derivatives) need not bring about a noticeable change in RI values of these molecules in the system with the nonpolar OV 1 stationary phase.

To find out interlaboratory differences the RI values of four β-adrenolytics (alprenolol, metoprolol, oxprenolol, and propranolol) and their TFA derivatives, found herein, were compared to the data obtained by *Yamaji et al.* [10] (Tables 2 and 3) on OV 101 (practically the same as OV 1 [11]) and OV 17 stationary phases.

As seen from Table 2, the differences in the values of the original drugs are, in accordance with expectation, higher on the more polar phase than on the nonpolar one, where they do not exceed, in the average, 1 %. This statement is

Table 2

Comparison of retention indices (RI) of four underivatized β-adrenolytics

Drug	OV 1 (OV 101)				OV 17			
	RI_1[a]	RI_2[b]	ΔRI[c]	ΔRI %[d]	RI_1[a]	RI_2[b]	ΔRI[c]	ΔRI %[d]
Alprenolol	1840	1826	+14	0.8	2044	2076	−32	1.6
Oxprenolol	1929	1900	+29	1.5	2169	2189	−20	0.9
Metoprolol	2041	2022	+19	0.9	2294	2336	−42	1.8
Propranolol	2152	2162	−10	0.5	2417	2487	−80	3.3
				0.93[e]				1.90[e]

a) Results of this work; *b*) data taken from [10]; *c*) $RI_1 - RI_2$; *d*) $\frac{RI_1 - RI_2}{RI_1} \cdot 100$; *e*) average value of ΔRI %.

Table 3

Comparison of retention indices (RI) of four trifluoroacetylated β-adrenolytics

Drug	OV 1 (OV 101)				OV 17			
	RI_1[a]	RI_2[b]	ΔRI[c]	ΔRI %[d]	RI_1[a]	RI_2[b]	ΔRI[c]	ΔRI %[d]
Alprenolol	1861	1860	+1	0.1	1975	1976	−1	0.1
Oxprenolol	1931	1931	0	0.0	2074	2073	+1	0.0
Metoprolol	2045	2049	−4	0.2	2202	2202	0	0.0
Propranolol	2165	2139	+17	0.8	2309	2349	−40	1.7
				0.28[e]				0.45[e]

The individual symbols are the same as in Table 2.

valid also in the case of TFA derivatives (Table 3). However, by derivatization the differences in RI values obtained in different laboratories decrease, regardless of the used stationary phase. In seven out of eight comparisons the RI values obtained herein differed by less than 1 % from those published by the Japanese authors.

Finally, it can be stated that for reproducibility of RI values the properties of the nonpolar OV 1 (OV 101) phase are more advantageous than those of OV 17. Further, by derivatization of β-adrenolytics with TFAA and HFBA the differences in RI values in the systems with OV 1 and OV 17 stationary phases decrease and so do the interlaboratory differences.

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