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INVESTIGATION OF RETENTION BEHAVIOR OF IMIDAZOLINE RECEPTOR LIGANDS ON MIXED-MODE HILIC STATIONARY PHASE

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ABSTRACT

Retention mechanism in the hydrophilic interaction liquid chromatography (HILIC) mode is complex and still a subject of many controversial interpretations. In order to describe the HILIC retention mechanism, different retention models can be employed. In this study, we investigated the partition mechanism for 7 imidazoline receptor ligands on the mixed mode HILIC stationary phase. Applicability of the assumed retention model in description of the HILIC retention behavior of the compounds was successfully demonstrated.

INTRODUCTION

The effect of mobile phase composition on the retention behavior can be described by different mathematical models, considering contribution of processes such as adsorption and partition to the investigated chromatographic mode [1, 2]. Complexity of retention processes in chromatography of hydrophilic interactions (HILIC) can involve different retention mechanisms and be reflected in a relationship between the retention factor of an analyte and the volume fraction of an aqueous modifier in mobile phase. The aim of this study was to investigate the influence of partition mechanism on the retention behavior of imidazoline-related compounds such, as amiloride, idazoxan, benazoline, moxonidine, cirazoline, tetrahydrozoline, oxymethazoline and clonidine [3] in the HILIC mode, using the mixed-mode HILIC column and the weakly acidic working conditions (pH 6).

EXPERIMENTAL

Amiloride hydrochloride, idazoxan hydrochloride, benazoline oxalate, moxonidine, cirazoline hydrochloride, tetrahydrozoline hydrochloride, oxymethazoline hydrochloride and clonidine hydrochloride were purchased from Sigma-Aldrich, St. Louis, MO, USA (Fig. 1).
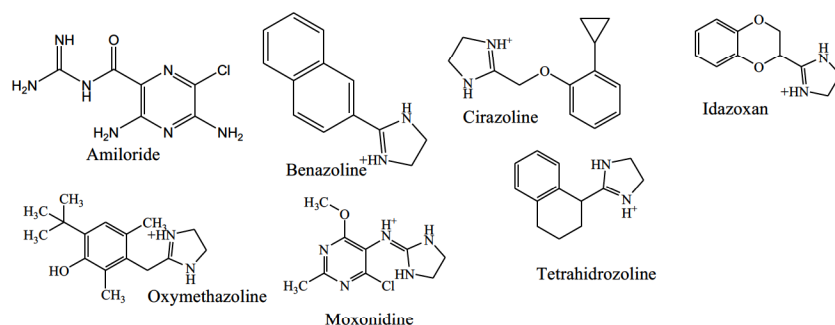
Figure 1. Dominant forms of investigated imidazoline receptor ligands at pH 6.

Acetonitrile (J.T. Baker, Deventer, The Netherlands) of HPLC grade and deionized water (TKA water purification system, Niederelbert, Germany) were used throughout this study. Ammonium acetate was obtained from Merck (Darmstadt, Germany). The HPLC analysis was performed using an Agilent Technologies 1200 HPLC system (Santa Clara, CA, USA). The analytical Acclaim Mixed-Mode HILIC-1 column 150mm × 4.6mm, silica particles size of 5μm (Thermo Fisher Scientific, Sunnyvale, CA USA) was used as stationary phase. Mobile phase consisted of acetonitrile and 20 mM ammonium acetate (pH 6). The flow rate was set to 1 mL min^{-1} and temperature to 25 °C. The UV detection was carried out at 225 nm.

The logk values were calculated from the obtained retention time (t_r) values for the three separate injections, using the below relationship:

$$\log k = \log ((t_r - t_0)/t_0) \qquad (1)$$

where t_0 is the retention time of the dead time marker, KNO_3.

In order to find minimal concentrations of the buffered eluent from which the HILIC mechanism started, the retention factors of the investigated compounds were correlated with the volume fractions of the buffered eluent for a wide range of these fractions. The turning point (φ_{min}) at which the HILIC mechanism started was calculated from the following equation:

$$\log k = a_1 + m_1 \cdot \varphi - m_2 \cdot \log \varphi \quad \rightarrow \quad \varphi_{min} = 0.434\, m_2/m_1 \qquad (2)$$

where a_1, m_1 and m_2 are the regression coefficients, and φ is the volume fraction of the buffered eluent. For low concentrations of aqueous phase ($\varphi < 0.02$), eq. 2 fails to properly describe the retention [2].

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The solute partitioning mechanism on stationary phase in the HILIC mode depends on volume fraction of an aqueous solvent (φ) in a binary mobile phase and it can be given by the following equation [1, 2]:

$$\log k = a_2 + m_3 \cdot \varphi = \log k_{ACN} + m_3 \cdot \varphi \qquad (3)$$

where a_2 holds for the logarithm of the retention factor of the solute in pure acetonitrile ($\log k_{ACN}$).

RESULTS AND DISCUSSION

The effect of volume fraction of the buffered eluent in the range of 0.10-0.75 was investigated in order to define its minimal concentration which influenced the beginning of the HILIC mode. The parameters of the relationship $\log k$ vs φ and the turning point (φ_{min}) for each compound are given in a Table 1.

Table 1. The regression coefficients and the turning point (φ_{min}) values of eq. 2 for the imidazoline receptor ligands.

Name of compounds	a_1	m_1	m_2	φ_{min}	R^2
Amiloride	-1.165	1.556	2.072	0.578	0.996
Idazoxan	-2.132	2.717	3.052	0.487	0.903
Tetrahydrzoline	-1.188	2.023	2.088	0.448	0.990
Benazoline	-1.222	2.286	2.145	0.407	0.980
Oxymethazoline	-1.804	3.400	2.577	0.329	0.984
Cirazoline	-1.329	2.514	2.144	0.370	0.981
Moxonidine	-0.527	0.824	0.912	0.480	0.986

At the next step of this study, the range 0.15-0.30 volume fraction of the buffered eluent showed high linearity of the relationship $\log k$ vs φ which was established. The influence of the volume fraction of the buffered eluent on the retention behavior of imidazoline derivatives was assessed with using eq. 3. In the determined chromatographic range, the retention factor values obtained from the isocratic process were linearly extrapolated to 100% volume fraction of acetonitrile ($\log k_{ACN}$). The obtained results are given in Table 2.

Table 2. Regression coefficients of eq. 3

Name of compounds	$\log k_{ACN}$	m_3	R^2
Amiloride	1.074	-2.350	0.995
Benazoline	1.179	-2.109	0.994
Cirazoline	1.020	-1.072	0.986
Idazoxane	0.811	-1.355	0.982
Oxymethazoline	1.041	-1.692	0.993
Moxonidine	0.422	-0.732	0.983
Tetrahydrozoline	1.091	-2.043	0.989

The high R^2 values (Table 2) confirmed that the retention data fit well to the partition model. Therefore the predominant contribution of the partition mechanism to the overall retention process is confirmed, affecting the retention behavior of the imidazoline-related compounds.

CONCLUSION
The obtained retention data demonstrate the influence of the partition mechanism on the retention behavior of the imidazoline receptor ligands in the HILIC mode.

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