Retention Characteristic of Ranitidine Hydrochloride on New Polymer-Based in Zwitter Ion Chromatography-Hydrophilic Interaction Chromatography Stationary Phases

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Summary: Two zwitterionic stationary phases with largely corroborated capacities were obtained by attachment sulfobetaine monomers ($H_2C=CHC_6H_4CH_2N^+(CH_3)_2$-$CH_2$-$SO_3^-$ and $H_2C=CHC_6H_4CH_2N^+(CH_3)_2$-$(CH_2)_5$-$SO_3^-$) onto a PS/DVB particles were investigated for chromatographic separation of ranitidine hydrochloride. The different chain lengths are used as an investigative tool for the retention behavior of pharmaceutical ranitidine hydrochloride. The retention behavior of ranitidine hydrochloride was examined with eluent at various ACN contain, buffer concentrations and pH. The separation mechanism is based on partitioning in reversed phase and ZIC ion exchange resulting in a mixed mode for the ranitidine hydrochloride. A direct calibration graph was constructed for ZIC_1 and ZIC_5 columns and it was found that the linear range (10-1000 $ng.ml^{-1}$), RSD% (0.41-1.55), LOD (1.55 and 2.56 $ng.ml^{-1}$), LOQ (5.17 and 8.53 $ng.ml^{-1}$), Recovery% (101.06 ± 1.15 and 100.47 ± 0.12) and E_{rel}% (1.666 ± 0.78 and 0.47 ± 0.12), respectively.

Keywords: Zwitterionic stationary phases; Sulfobetaine; Ion exchange; Ranitidine hydrochloride; Reversed phase.

Introduction

Hydrophilic interaction liquid chromatography (HILIC) is a rapidly growing alternative to reversed phase chromatography for the separation of hydrophilic compounds under conditions of high concentrations of organic solvents on hydrophilic supports. The selectivity observed is comparable to NPLC. Alpert [1] proposed a separation mechanism for HILIC based on partitioning between a water-enriched layer on the stationary phase surface and a mainly organic mobile phase. Zwitterionic stationary phases can serve as highly hydrophilic supports with strong water enrichment at the surface. Ranitidine hydrochloride also called Zantac (*N*-[2-[[[5-[(dimethylamino) methyl]furan-2-yl]methyl]thio]ethyl]- *N*-methyl-2-nitroethene-1,1-diamine hydrochloride, RANH, Fig. 1) is H_2-histamine receptor antagonist that is widely used for the treatment for anti-ulcer agent [2].

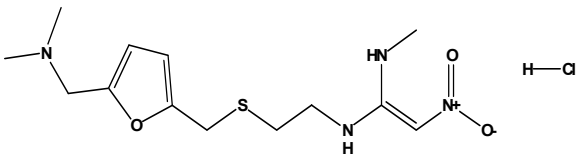

Fig. 1: Structure formulae of (RANH).

Notwithstanding the availability of numerous works of separation RANH in HPLC [3-8], no investigation has been carried out for the retention characteristic of RANH in ZIC-HILIC mode. The second goal of our work is to investigate the influence of chain length between the charges (one methylene group ZIC_1 and five methylene groups ZIC_5). Recently, Rasheed et al. [9] study retention behavior of eight pharmaceutical compounds using four ZIC columns. This study involves the influence of the different spacer lengths between charged functional groups in ZIC-HILIC columns. They found the separation of the pharmaceuticals relied on ion exchange interactions with the ZIC columns. In previous works investigating the separation of the pharmaceutical-metal complexes [10, 11] and therefore, they proved that the ZIC-HILIC columns are able to separate desferrioxamine-metal and trifluoperazine hydrochloride-metal complexes by IC-ICP-AES.

HILIC at present attracts much attention because it solves many problems of previously difficult separations. It has been successfully applied to the analysis of carboxylic acid [12], inorganic anions [13], sugar [14], saccharides [15] and dansyl amino acids [16] by liquid chromatography. An advance in the understanding of retention mechanisms during HILIC separations increases the range of possible applications of liquid chromatography. The third goal is to introduce simple method for the determination of RANH in pure and pharmaceutical (Zantac) samples.

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Experimental

A Merck-Hitachi HPLC system with L-6200 gradient pump and L-4200 ultraviolet–visible detector, a 20 µL injection loop was used. The pH measurements were conducted on pH 740 (WTW). The N2000 Photographic Data Workstation software was used to control my chromatography and analyze the data. The detection of RANH was carried by using ultraviolet region at a wavelength of 230 nm. The chromatographic conditions are summarized in Table-1. The stationary phases (ZIC_1 and ZIC_5) used for the RANH separation were a self-made via grafted sulfobetaine monomers ($H_2C{=}CHC_6H_4CH_2N^+(CH_3)_2\text{-}CH_2\text{-}SO_3^-$ and $H_2C{=}CHC_6H_4CH_2N^+(CH_3)_2\text{-}(CH_2)_5\text{-}SO_3^-$) [10, 11, 13, 16-18] onto the PS/DVB using PEEK columns (100 mm × 4 mm I.D.). The cation exchange columns was homemade via a grafting reaction adopted from patent [18] and had a capacity of 48 µeq g^{-1} using PEEK columns (100 mm × 4 mm I.D.). The detailed procedure of the grafting reaction has been described by Raskop et al. [18]. Ranitidine hydrochloride was purchased from Sigma. Acetic acid (HAc) was purchased from BDH. Sodium acetate (NaOAc) was purchased from Fluka. Acetonitrile (ACN) HPLC grade (≥99.93%) was purchased from Aldrich. The ZIC_1 and ZIC_5 columns have capacity 432 and 488 µeq g^{-1} [13], respectively. Thirteen tablets for each of the Zantac samples were crushed and the equivalent to about 20 mg of RANH was dissolved an adequate size of water and transferred into a 100 mL volumetric flask and diluted to the mark with water. Subsequently, the solution was filtered by millipore filters (0.45 µm).

Table-1: The chromatographic conditions of proposed method.

Chromatographic conditions	
Detector	**230 nm**
Injection volume µL	**20 µL**
Flow rate mL/min	**0.75 mL/min**
Temperature °c	**45 °c**
Eluent	**ACN/NaOAc/HAc Buffer**

Results and Discussion

Separation of ranitidine hydrochloride

RANH was chosen as test pharmaceutical for a study on their retention mechanism in HILIC mode by applying a NaOAc/HAc buffer mobile phase with varying ACN content on the two of ZIC-HILIC columns. The chromatogram is shown in Fig 2. The chromatogram was achieved at 80% ACN and 40 mM (pH 4.75) of NaOAc/HAc buffer. Mobile phase compositions are changed systemic by variation of the ACN content from 20% to 90% (v/v); the concentration of the eluent from 20 to 100 mM and its pH from 3.5 to 5.5, in order to get a clue about the separation properties of the individual stationary phases and thus about the separation mechanism. The anion exchange column has been used as reference point for solely ionic retention behavior.

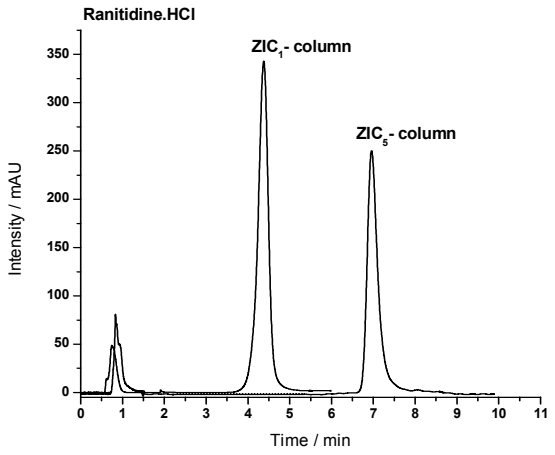

Fig. 2: Chromatogram for RANH separated on ZIC_1 and ZIC_2 columns.

It should be noted that retention of RANH in ZIC_5 column exhibits the highest retention in comparison with the behavior of RANH in ZIC_1 column. The reason for this inevitably the methylene group between charged groups in ZIC-HILIH columns[13]. There seems to be a special geometrical alignment of sulfobetaine groups in the ZIC_5 exchanger as they exhibit the highest pharmaceutical retention. These various interactions are stemmed from the different flexibility of the sulfobetaine chains influencing their ability to form intra- and intermolecular ion pairs. Thus, the spacer length between the charges of zwitterionic exchangers should show an influence on the retention of pharmaceuticals.

Effect of ACN Content on Retention of Ranitidine Hydrochloride

In a previous study [9], the retention for the eight pharmaceuticals separation (Deferoxamine mesylate, Thia-mine. HCl, Diclofenac sodium, Cyclopentolate. HCl, Dexamethasone sodium phosphate, Tetracaine. HCl, Pilocarpine. HCl, Chloramphenicol) increased or decreased with increasing ACN content in ZIC-HILIC mode. The reason for this difference in the behavior of the model pharmaceuticals when varying ACN content in the eluent is due to the hydrophilicity of pharmaceuticals, as it is clear from the log P_{ow} values of the pharmaceuticals. Consequently, the pharmaceuticals exhibit two

behavior hydrophilic (HILIC) and hydrophobic (RP) with increasing ACN content in the eluent. The reason for this difference in the behavior is due to the hydrophilicity of the pharmaceuticals. RANH exhibits its hydrophobic (RP) behavior for both ZIC_1 and ZIC_5 columns (Fig. 3). The reason for that goes back to the log P_{ow} (0.99) [19] value of RANH. It is worth to be mentioned that the retention of RANH shows the highest retention in ZIC_5 columns is due to the space length between charged groups.

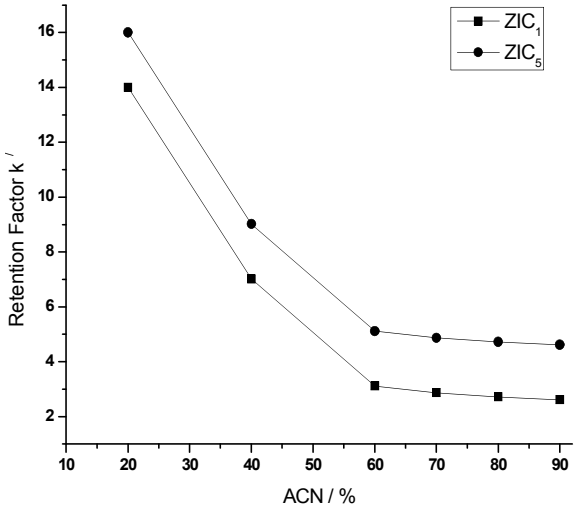

Fig. 3: Effect of ACN content on retention of RANH.

Effect of eluent concentration on retention of ranitidine hydrochloride

In general, the retention of analyte in ZIC-HILIC mode increased with increasing eluent concentration which leads to a deactivation of intramolecular ion pairs. Thus strengthen the linearization of the functional groups of the column although the presence of ACN [12]. The retention time of the pharmaceuticals in ZIC-HILIC columns increased or decreased with increasing NaOAc/HAc buffer concentration [9]. The reason for that attributes it to anion and cation exchange interactions. Fig. 4 illustrates the retention factor of RANH decreased when the NaOAc/HAc buffer was increased from 20 to 100 mM while holding pH at 4.75 and ACN at 80%. The slopes (0.3069 and 0.3236) were obtained from Fig. 4a it seems that such a slope measured for conventional ion exchange columns [20]. The question that arises is what "real" separation mechanism? RANH have a different picture when increasing NaOAc/HAc buffer concentration the retention decreased and, therefore, we believe this is due to two reasons. Firstly, the hydrophilicity of RANH and secondly, the core material (PS/DVB) of columns. The pka value (8.2) and the isoelectric points (13.24) [21] of RANH. Thereby, the examined RANH should be in cationic. A similarly constructed cation exchanger based on the same core material and using sulfonic acid as a functional group and the same grafting reaction showed the expected negative slope [18]. Therefore, the separation of the RANH relied on the cation exchange with the ZIC-HILIC columns (Fig. 4b).

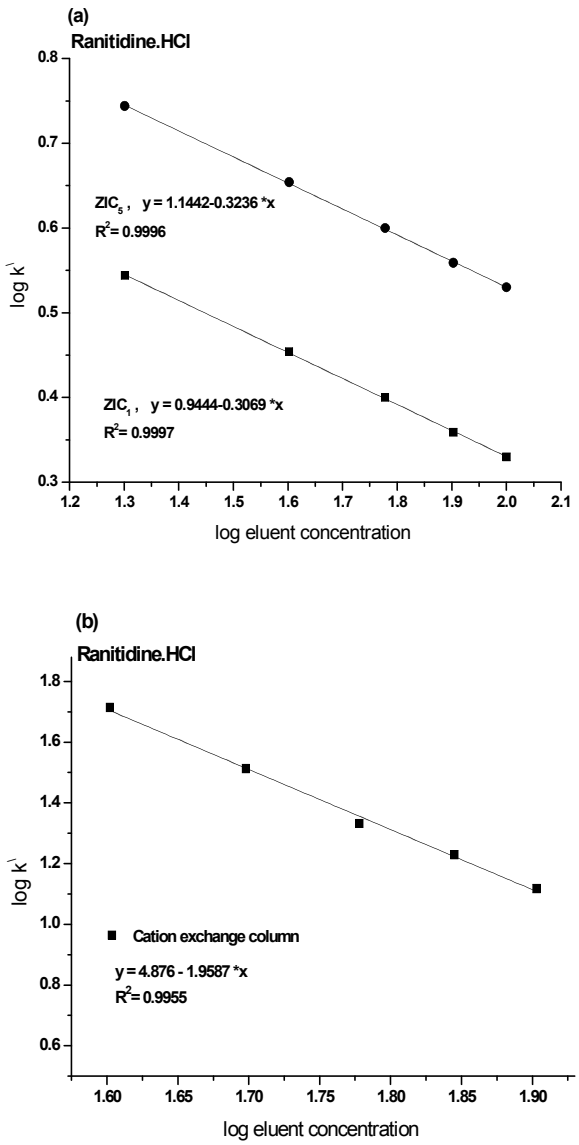

Fig. 4: Effect of eluent concentration on retention of RANH (a) ZIC_1 and ZIC_5 columns (b) Cation exchange column.

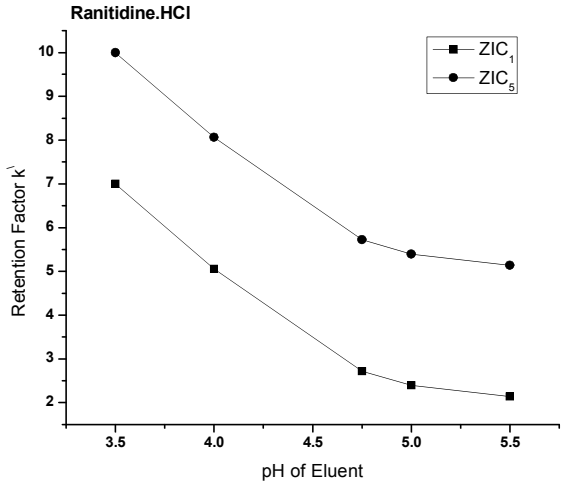

Fig. 5: Effect of eluent pH on retention of RANH.

Effect of eluent pH on retention of ranitidine hydrochloride

To complete clue of RANH separation under HILIC-mode, the eluent pH has to be varied. The retention factor of RANH decreased when the eluent pH was increased from 3.5 to 5.5 while holding NaOAc/HAc buffer concentration at 40 mM and ACN at 80% as shown in Fig. 5. RANH with an isoelectric point 13.24, the retention time decreased on ZIC_1 and ZIC_5 columns due to the protonation of the amino group in RANH.

Calibration graph

A calibration graph of RANH established by plotting the area versus concentration of RANH and shows the range concentration (10-1000 ng mL^{-1}) of ZIC_1 and ZIC_5 columns (Fig. 6).

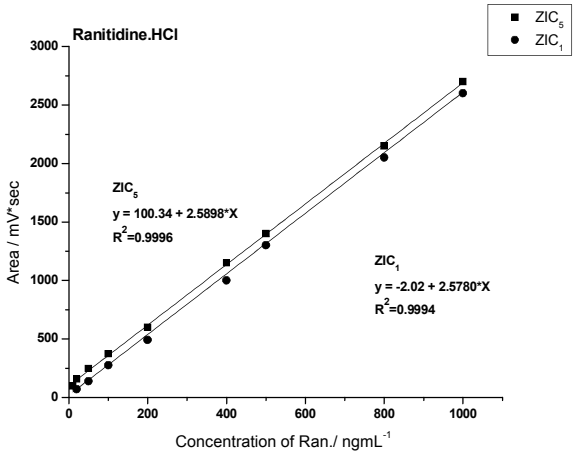

Fig. 6: Calibration graph for RANH.

Statistical data analysis

The direct calibration graph for the direct determination of RANH under ZIC-HILIC conditions was constructed and the statistical results are illustrated in Table-2.

The same-day and the day-to-day accuracy and precision were examined and calculating by recovery % and RSD %, respectively. The low relative standard deviation values and the high recovery values refer that the proposed method is precise (Table-3).

Table-2: Analytical characteristics of result.

Parameter	ZIC_1-columns	ZIC_5-columns
Linearity ($ng.ml^{-1}$)	10-1000	10-1000
Regression equation	y = -2.02 + 2.5780* x	y = 100.34 + 2.5898* x
Correlation coefficient (r)	0.9997	0.9998
Coefficient of determination (r^2)	0.9994	0.9996
Limit of detection (LOD) ($ng.mL^{-1}$)	2.56	1.55
Limit of quantification (LOQ) ($ng.mL^{-1}$)	8.53	5.17

Table-3: Precision and accuracy of the proposed method.

Same-Day Analysis n=5					Day-to-Day Analysis n=5			
ZIC_1 column								
RAN Taken ($ng.mL^{-1}$)	RAN Found ($ng.mL^{-1}$)	% Rec.	% Erel.	%RSD	RAN Found ($ng.mL^{-1}$)	% Rec.	% Erel.	%RSD
100	100.33	100.33	0.33	0.82	101.88	101.88	1.88	0.87
500	502.55	100.51	0.51	0.44	506.22	101.24	1.24	0.41
700	704.00	100.57	0.57	0.77	702.78	100.39	0.34	0.71
					ZIC_5 column			
100	102.00	102.00	2.00	0.52	101.5	101.50	1.50	0.55
500	498.88	99.77	-0.22	1.26	502.0	100.40	0.40	1.20
700	710.00	101.42	1.42	1.55	713.0	101.85	1.85	0.97

Table-4: Application of proposed method for determination of RANH in pharmaceutical preparation.

Name of pharmaceutical	Manufacturer	Stated conc. (mg)	Found direct calb. (mg)	Rec. %	RSD % n=5	E_{rel} %
		ZIC_1 column				
HISTAC	RANBAXY India	150	147.22	98.14	0.82	-1.85
		150	150.44	100.29	0.39	0.29
ACID REDUCER	Allegiant Health Canada	150	147.77	98.51	0.72	-1.48
		ZIC_5 column				
RANAC	PIONEER Co. Iraq	150	146.87	97.91	0.87	-2.08
		150	150.90	100.60	0.33	0.60
		150	146.87	97.91	0.67	-2.08

Determination of RANH in Zantac

The proposed method was applied successfully to the determination of RANH in three of the pharmaceutical preparations containing RANH (tablet) with stated concentration of 150 mg per unit the results obtained are given in Table 4.

Conclusion

The present work includes the development of ZIC-HILIC method for the determination of RANH in pharmaceutical samples. The sulfobetaine columns with one and five methylene groups between the charged groups were used a versatile separation tools with the advantage of activating at least three different retention modes by varying the mobile phase conditions. This paper exhibits how the RANH interact with new zwitterionic stationary phases ZIC_1 and ZIC_5. It was found, that the ZIC_5 column exhibits higher retention time with RANH in comparison to ZIC_1 column. It may be the cause due to being a geometrical alignment of the ZIC_5 column. The experimental data showed that both hydrophobic and cation exchange behaviors are active as a retention mechanism. The developed method was successfully applied to the determination of RANH in pharmaceutical samples.

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