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Famotidine determination in pure and pharmaceutical formulations by zwitterionic chromatography-hydrophilic interaction liquid chromatography

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Abstract : Sulfobetaine exchanger(ZIC-HILIC$_1$) with largely capacity(432 μeq g^{-1})was obtained by attachment sulfobetaine monomer with one methylene between charges (H_2C=$CHC_6H_4CH_2N^+(CH_3)_2$-CH_2-SO_3^-) onto a PS/DVB particles was investigated for chromatographic separation of famotidine.The retention behavior of famotidine was investigated with eluent at various Acetonitrile contain, sodium acetate buffer concentrations and pH. The separation mechanism is according to hydrophilic interaction liquid chromatography and cation exchange which leads to a mixed mode for the famotidine. A calibration graph it was created for ZIC-HILIC$_1$exchanger and it was found that the linear range (20-800 ng.ml^{-1}), RSD% (0.72-1.76), LOD (4.10 ng.ml^{-1}), LOQ (13.64 ng.ml^{-1}).
Keywords : Zwitterionic chromatography, Sulfobetaine stationary phases, famotidine, Retention mechanism, ion exchange.

Introduction

Hydrophilic interaction liquid chromatography (HILIC) is a rapidly growing alternative to RPLC 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. Famotidine, *N2*-(aminosulphonyl)-3-[[[2-[(diaminomethylene) amino] thiazol-4-yl]methyl]thio]propanamidine, FAM, Figure 1) is H_2-histamine receptor antagonist that is widely used for the treatment for ulcer agent in duodenal and gastric [2].

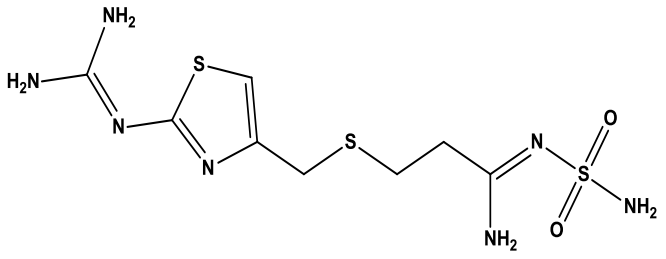

Figure 1: Structure formulae of (FAM).

In spite of the availability of numerous works of separation FAM in HPLC [3-8], no investigation has been carried out for the retention characteristic of FAM in ZIC-HILIC mode. Recently, Rasheed et al.[9] study retention behavior of pharmaceutical compounds using four ZIC-HILIC 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-metalcomplexes 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 second goal is to introduce simple method for the determination of FAM in pure and pharmaceutical (FAMOSAM and Ulceran)formulations.

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 FAM was carried by using ultraviolet region at a wavelength of 282 nm. The exchanger ZIC-HILIC$_1$ used for the FAM separation were a self-made via grafted sulfobetaine monomer (H_2C=$CHC_6H_4CH_2N^+(CH_3)_2$-CH_2-SO_3^-) [13] onto the PS/DVB using PEEK columns (100 mm × 4 mm I.D.). The detailed procedure of the grafting reaction has been described by Raskop et al. [17].FAM was purchased from Sigma. Acetic acid was purchased from BDH. Sodium acetate (NaOAc) was purchased from Fluka. Acetonitrile (ACN) HPLC grade (≥99.93%) was purchased from Aldrich. The ZIC-HILIC$_1$exchanger has capacity 432µeq g^{-1}[11, 13]. Thirteen tablets for each of the FAMOSAM and Ulceran formulations were crushed and the equivalent to about 10 mg of FAMwas 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).

Results and discussion

Separation of Famotidine

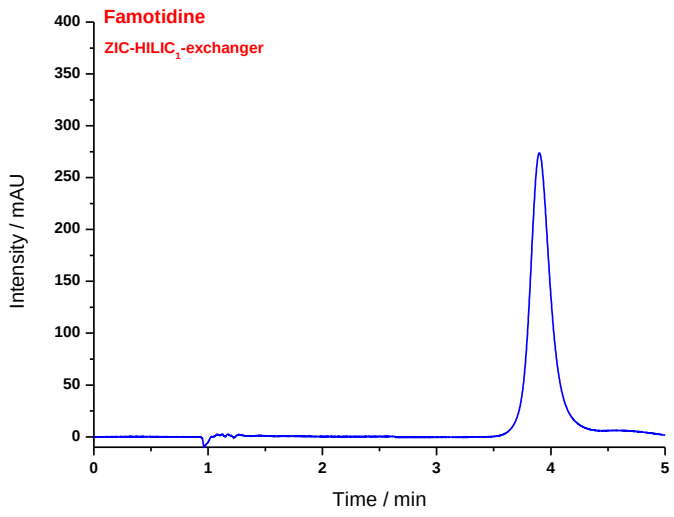

Figure 2: Chromatogram for FAM separated on ZIC-HILIC$_1$exchanger.

The separation of FAM was study in HILIC-mode by applying a sodium acetate mobile phase with varying ACN content on the ZIC-HILIC$_1$exchanger. The chromatogram is shown in Figure 2. The chromatogram was accomplished at 40 mM (pH 4.75) of sodium acetate and 80% ACN. Mobile phase compositions are changed methodology by variation of the ACN content from 20% to 90%; the concentration of the eluent from 20 to 100 mM and its pH from 4 to 5.5, in order to get aidea about the separation characteristics of the individual column and thus about the separation mechanism.

Effectof ACN content

Rasheed et al. [9] investigated the retention for the pharmaceuticals separation increased or decreased with increasing ACN content in ZIC-HILIC mode. Accordingly, the pharmaceuticals show two behavior hydrophobic (RP) and hydrophilic (HILIC) with increasing CAN content in the mobile phase. The hydrophilicity of the pharmaceuticals plays a key role for this difference in the behavior. The log P_{ow} value of FAM is (-2)[18]and, therefore, FAM exhibits HILIC behavior for ZIC-HILIC$_1$exchanger(Figure 3).

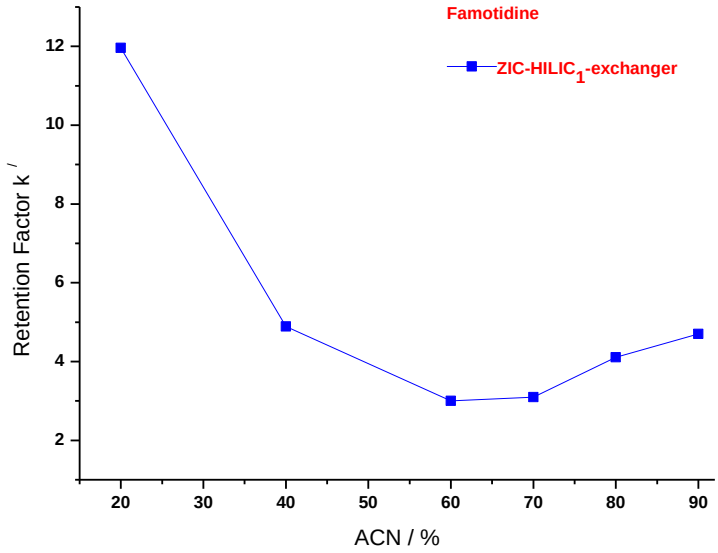

Figure 3: Effect of ACN content on retention of FAM.

Effect of eluent concentration

Mostly, the retention of solute 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 stationary phase although the presence of ACN [12]. The retention of the pharmaceuticals in ZIC-HILIC stationary phases decreased or increased with increasing buffer concentration [9]. The reason for that attributes it to cation exchange interaction. Figure 4 exhibits the retention of FAM decreased when the NaOAc buffer was increased from 20 to 100 mM while holding pH at 4.75 and ACN at 80%. The slope (0.9123) were obtained from Figure 4 it seems that such a slope measured for conventional ion exchange columns [19].

The question now is what "real" separation mechanism? FAM have a different picture when increasing buffer concentration the retention decreased and, therefore, we believe this is due to two reasons. The hydrophilicity of FAM and the core material of exchanger. The pka value (6.7) and the isoelectric points (8.8) [20] of FAM. Subsequently, FAM should be in cationic form. And therefore, the separation of the FAM relied on the cation exchange with the ZIC-HILIC stationary phase.

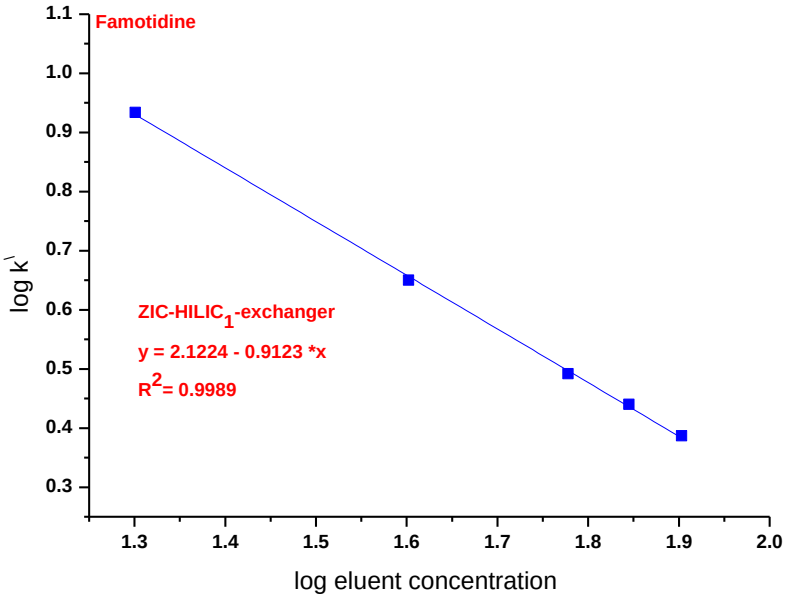

Figure 4: Effect of buffer concentration on retention of FAM.

Effectof eluent pH

To complete the idea of separation mechanism of FAM, the eluent pH has to be varied. The retention of FAM increased when the eluent pH was decreased from 3.5 to 5.5 while holding sodium acetate concentration at 40 mM and ACN at 80% as shown in Figure 5. FAM with an isoelectric point 8.88, the retention decreased on ZIC-HILIC$_1$exchangerdue to the protonation of the amino group in FAM.

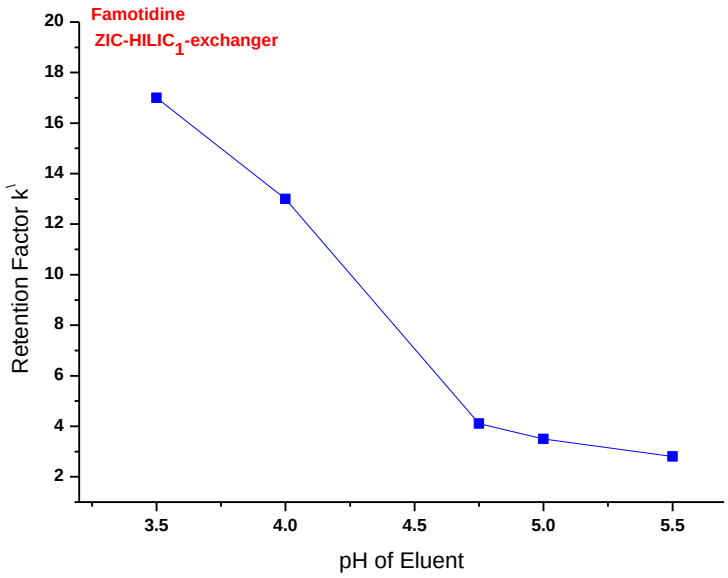

Figure 5: Effect of eluent pH

Calibration graph

A calibration graph of FAM established by plotting the area versus concentration ofFAM and exhibits the range concentration (20-800 ng mL^{-1}) of ZIC-$HILIC_1$exchanger(Figure 6).

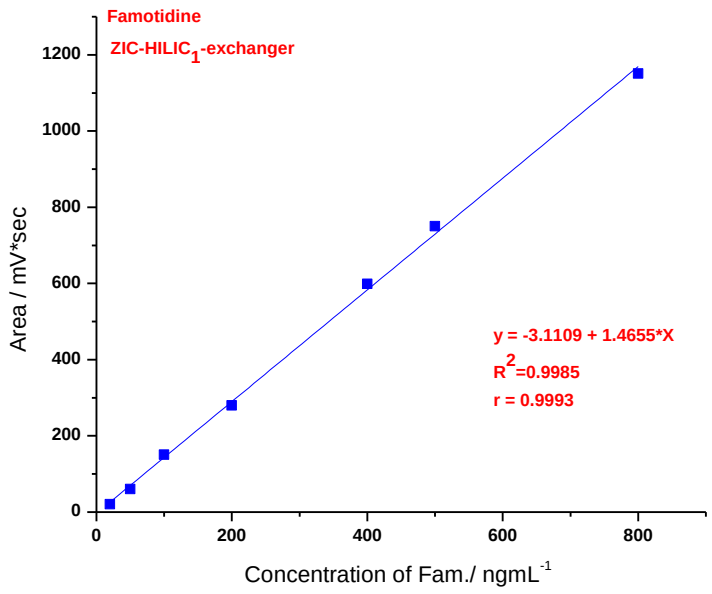

Figure 6: Calibration graph for FAM.

Statistical data analysis

The direct calibration graph for the direct determination of FAM under HILIC conditions was constructed and the statistical results are illustrated in Table 1.

Table 1: Analytical characteristics of result.

Parameter	**ZIC-$HILIC_1$-exchanger**
Linearity ($ng.ml^{-1}$)	20-800
Regression equation	y = -3.1109 + 1.4655*x
Correlation coefficient (r)	0.9993
Coefficient of determination (r^2)	0.9985
Limit of detection (LOD) ($ng.mL^{-1}$)	4.10
Limit of quantification (LOQ) ($ng.mL^{-1}$)	13.64

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 2).

Table 2 : Precision and accuracy of the proposed method.

	Same-Day Analysis n=5				**Day-to-Day Analysis n=5**			
	ZIC-HILIC$_1$exchanger							
FAM Taken (ng.mL^{-1})	**FAM Found (ng.mL^{-1})**	**% Rec.**	**% Erel.**	**%RSD**	**FAM Found (ng.mL^{-1})**	**% Rec.**	**% Erel.**	**%RSD**
75	75.88	101.17	1.17	1.76	75.55	100.73	0.73	1.33
120	119.76	99.80	-0.20	1.66	119.31	99.42	-0.57	1.46
400	402.12	100.53	0.53	0.98	402.77	100.69	0.69	0.72

Determination of FAM in pharmaceutical formulations

The proposed method was applied successfully to the determination of FAM in two of the pharmaceutical formulations; the results obtained are given in Table 3.

Table 3: Application of proposed method for determination of FAM in pharmaceutical formulations.

Name of pharmaceutical	**Manufacturer**	**Stated conc. (mg)**	**Found direct calb. (mg)**	**Rec. %**	**RSD % n=5**	**E_{rel} %**
FAMOSAM	Samarra drugs factory-IRAQ	40	41.55	103.87	1.23	3.87
Ulceran	Medochemie LTD, Limassol-Cyprus	20	19.74	98.70	0.72	-1.30

Conclusion

The data presented in this article includes the development of HILIC method for the determination of FAM in pharmaceutical formulations. The zwitterionic stationary phase with one methylene groups between the charged groups were used a versatile separation tool with the advantage of activating at least three different retention modes by varying the eluent conditions. This artical show how FAM interact with new zwitterionic-exchanger ZIC-HILIC$_1$. It was found, that the ZIC-HILIC$_1$exchangerexhibits higher retention time with FAM. It may be the cause due to being a geometrical alignment of the ZIC-HILIC$_1$exchanger. The experimental data showed that both HILIC and cation exchange behaviors are active as a retention mechanism. The developed method was successfully applied to the determination of FAM in pharmaceutical formulations.

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