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Study on the retention behavior of famotidine in hydrophilic interaction liquid chromatography

Marwah Adnan Abbas, Ashraf Saad Rasheed*

Department of Chemistry, College of Science, University of Baghdad, Al-Jadriya campus, 10071 Baghdad, Iraq

Abstract : Zwitterionic stationary phase with largely capacity was obtained by attachment sulfobetaine monomer ($H_2C=CHC_6H_4CH_2N^+(CH_3)_2$-$(CH_2)_5$-$SO_3^-$) onto a PS/DVB particles was investigated for chromatographic separation of famotidine. The retention behavior of famotidine was examined with eluent at various ACN contain, buffer concentrations and pH. The separation mechanism is based on partitioning in hydrophilic interaction liquid chromatography and ZIC ion exchange resulting in a mixed mode for the famotidine. A direct calibration graph was constructed for ZIC_5 stationary phase and it was found that the linear range (20-800 $ng.ml^{-1}$), RSD% (0.74-2.03), LOD (3.56 $ng.ml^{-1}$), LOQ (11.87 $ng.ml^{-1}$).
Keywords: Zwitterionic chromatography, Sulfobetaine stationary phases, famotidine, Retention mechanism, ion exchange.

1. 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. 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].

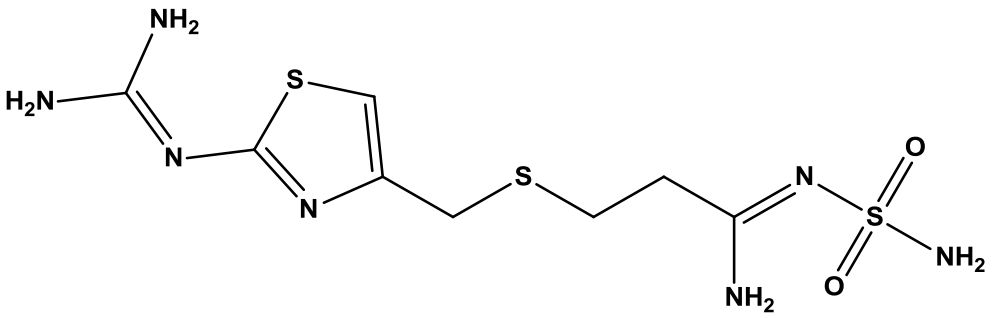

Figure 1: Structure formulae of (FAM).

Notwithstanding 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 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-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) samples.

2. 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 chromatographic conditions are summarized in Table 1. The stationary phase ZIC_5 used for the FAM separation were a home-made via grafted sulfobetaine monomer ($H_2C{=}CHC_6H_4CH_2N^+(CH_3)_2{-}(CH_2)_5{-}SO_3^-$) [11, 16-18]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_5 column has capacity 488 µeq $g^{-\backslash}$[10, 11, 16]. Thirteen tablets for each of the FAMOSAM andUlceran samples were crushed and the equivalent to about 10 mg of FAM 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	282 nm
Injection volume µL	20 µL
Flow rate mL/min	0.75 mL/min
Temperature ^{o}c	45 ^{o}c
Mobile phase	Acetonitrile / Sodium acetate

3. Results and discussion

Separation of Famotidine

FAM was chosen as test pharmaceutical for a study on their retention mechanism in HILIC mode by applying a NaOAc mobile phase with varying ACN content on the ZIC-HILIC column. The chromatogram is shown in Figure 2. The chromatogram was achieved at 80% ACN and 40 mM (pH 4.75) of NaOAc.

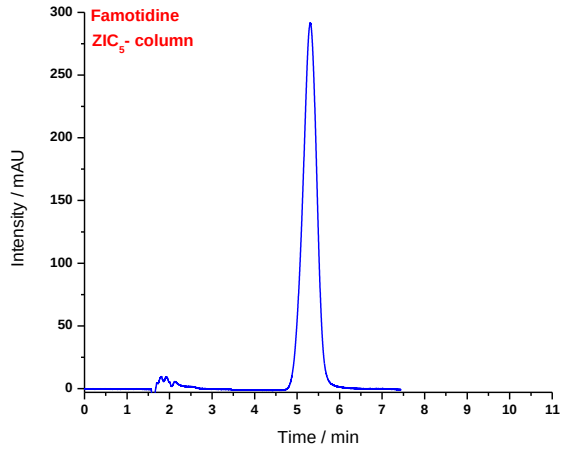

Figure 2: Chromatogram for FAM separated on ZIC_5 stationary phase.

Mobile phase compositions are changed systemic by variationof the ACN content from 20% to 90% (v/v); the concentration of the eluent from 20 to 100 mM and its pH from 4 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.

Influence of ACN content on retention of FAM

In a previous study [9], the retention for the eight 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 decreasing water content in the eluent. The reason for this difference in the behavior is due to the hydrophilicity of the pharmaceuticals. FAM shows hydrophilic FAM shows hydrophilic (HILIC) behavior for ZIC_5 stationary phase (Figure 3). The reason for that due to the log P_{ow} (- 2) [19] value of FAM.

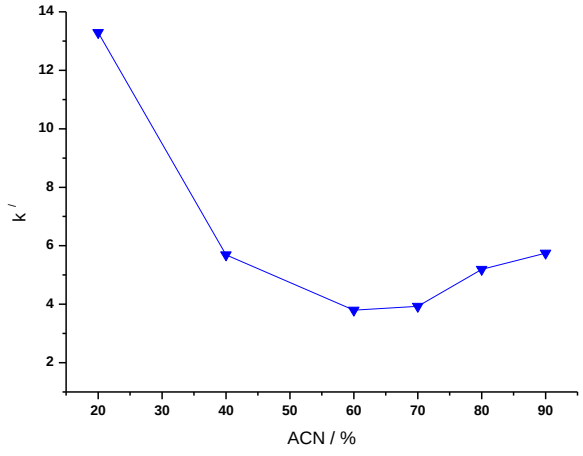

Figure 3: Influence of ACN content on retention of FAM.

Influence of eluent concentration on retention of FAM

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 and anion exchange interactions.

Figure 4 illustrates the retention factor 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 (0.9450) were obtained from Figure 4 it seems that such a slope measured for conventional ion exchange columns [20]. 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 stationary phase. The pka value (6.7) and the isoelectric points (8.8) [21] 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.

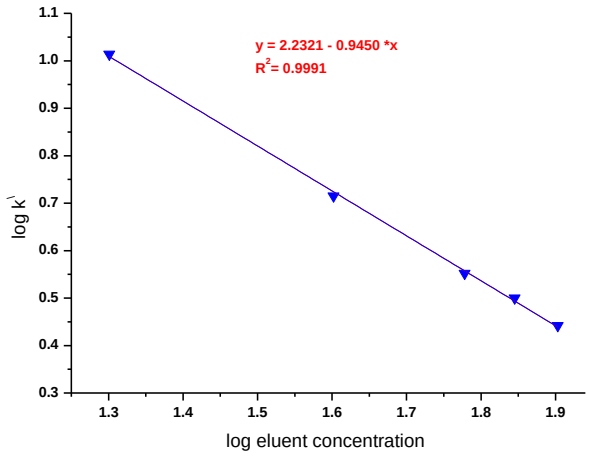

Figure 4: Influence of buffer concentration on retention of FAM.

Influence of 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 4 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_5 stationary phase due to the protonation of the amino group in FAM.

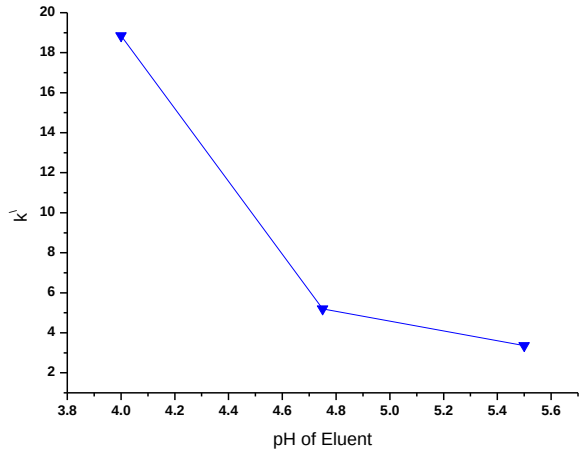

Figure 5: Influence of eluent pH

Calibration graph

A calibration graph of FAM established by plotting the area (mV*sec) versus concentration (ng.mL^{-1})of FAM and exhibits the range concentration (20-800 ng mL^{-1}) of ZIC_5 stationary phase (Figure 6).

Statistical data analysis

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

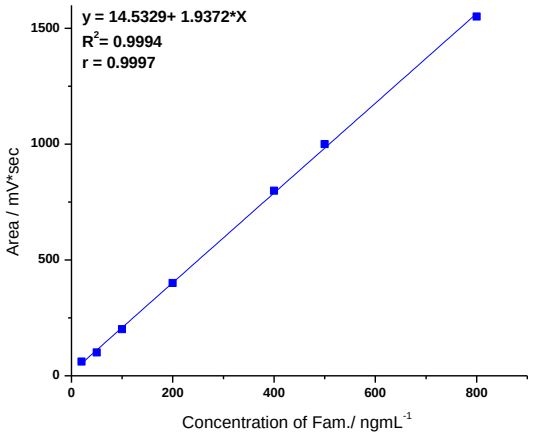

Figure 6: Calibration graph for FAM.

Table 2: Analytical characteristics of result.

Parameter	**ZIC_5- stationary phase**
Linearity (ng.ml^{-1})	20-800
Regression equation	y = 14.5329+ 1.9372*x
Correlation coefficient (r)	0.9998
Coefficient of determination (r^2)	0.9997
Limit of detection (LOD) (ng.mL^{-1})	3.56
Limit of quantification (LOQ) (ng.mL^{-1})	11.87

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 3:Precision and accuracy of the proposed method.

Same-Day Analysis* *n=5				
FAM Taken (ng.mL^{-1})	**FAM Found (ng.mL^{-1})**	**% Rec.**	**% Erel.**	**%RSD**
75	76.50	102.00	2.00	1.91
120	120.89	100.74	0.74	2.03
400	403.55	100.88	0.88	1.62
Day-to-Day Analysis *n=5*				
	76.18	101.57	1.57	1.86
	120.73	100.60	0.60	0.87
	403.73	100.93	0.93	0.74

Determination of FAM inpharmaceutical samples

The proposed method was applied successfully to the determination of FAM in two of the pharmaceutical preparations; the results obtained are given in Table 4.

Table 4: Application of proposed method for determination of FAM in pharmaceutical samples.

Name of pharmaceutical	Manufacturer	Stated conc. (mg)	Found direct calb. (mg)	Rec. %	RSD % n=5	E_{rel} %
FAMOSAM	S.D.I.-IRAQ	40	41.22	103.05	1.11	3.05
Ulceran	Medochemie LTD, Limassol-Cyprus	20	19.55	97.75	0.88	-2.25

Conclusion

The current study includes the development of HILIC method for the determination of FAM in pharmaceutical samples. The zwitterionic stationary phase with five 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 zwitterionic stationary phase ZIC_5. It was found, that the ZIC_5 column exhibits higher retention time with FAM. It may be the cause due to being a geometrical alignment of the ZIC_5 stationary phase. 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 samples.

References

1. Alpert, A.J., Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. Journal of Chromatography A1990, 499, 177-196.
2. Pharmacopoeia, B., CD–ROM; Version 12. London. The Stationery Office2008.
3. Haque, A., Shahriar, M., Parvin, M.N., Islam, S., Validated RP-HPLC method for estimation of ranitidine hydrochloride, domperidone and naproxen in solid dosage form. Asian Journal of Pharmaceutical Analysis2011, 1(3), 59-63.
4. Naresh, M., Ganesh, T., Biswal, A., Nagarjuna, R., RP-HPLC Method development and validation studies of Ranitidine hydrochloride and Domperidone. IJCNS2013, 1(1), 25-8.
5. Zendelovska, D., Stafilov, T., Development of an HPLC method for the determination of ranitidine and cimetidine in human plasma following SPE. Journal of pharmaceutical and biomedical analysis2003, 33(2), 165-173.
6. Rustum, A.M., Rapid and sensitive HPLC determination of ranitidine in plasma. Application to pharmacokinetics study. Journal of liquid chromatography1988, 11(11), 2315-2335.
7. Enein, H.Y.A., Islam, M.R., Liquid chromatographic analysis of ranitidine hydrochloride in pharmaceutical preparations. Toxicological & Environmental Chemistry1990, 29(1), 47-51.
8. Farthing, D., Brouwer, K.L., Fakhry, I., Sica, D., Solid-phase extraction and determination of ranitidine in human plasma by a high-performance liquid chromatographic method utilizing midbore chromatography. Journal of Chromatography B: Biomedical Sciences and Applications1997, 688(2), 350-353.
9. Rasheed, A.S., Influence of capacity on the retention characteristics in Zwitter Ion Chromatography (ZIC) and ZICHydrophilic Interaction Chromatography (HILIC) onfour different sulfobetaine stationary phases. PhD Thesis, Philipps-Universität Marburg2014.
10. Bashaer Abbas, A.-P., Ashraf Saad, R., ICP Spectrometric–Vis Separation of Cerium (IV)–Desferal Complex Using 4-Vinylbenzyl-Dimethylammonio Pentanesulfonate Zwitterionic Stationary Phase. Journal of Al-Nahrain University - Science2016, 19(2), 25-32.
11. Rasheed, A.S., Seubert, A., Separation of Metal–Trifluoperazine Hydrochloride Complexes Using Zwitterionic Ion Chromatography (ZIC) Coupled Online with ICP-AES. Current Pharmaceutical Analysis2017, 12, 1-6.

12. Rasheed, A.S., Al-phalahy, B.A., Seubert, A., Studies on Behaviors of Interactions Between New Polymer-based ZIC-HILIC Stationary Phases and Carboxylic Acids. Journal of Chromatographic Science2017, 55(1), 52-59.
13. Rasheed, A.S., Seubert, A., Influence of Capacity on the Retention and Selectivity of Inorganic Ions Separation Over a Homologous Series of Sulfobetaine Based Stationary Phases in Zwitterionic Ion Chromatography. Current Chromatography2016, 3(1), 4-11.
14. Palmer, J.K., A versatile system for sugar analysis via liquid chromatography. Analytical Letters1975, 8(3), 215-224.
15. Linden, J.C., Lawhead, C.L., Liquid chromatography of saccharides. Journal of Chromatography A1975, 105(1), 125-133.
16. Al-Phalahy, B.A., Muhamad, Y.H., Rasheed, A.S., Zwitterionic Ion Chromatography of Dansyl Amino Acids with 4-Vinylbenzyl Dimethyl Ammonio Pentanesulfonate as Stationary Phase. Asian Journal of Chemistry2016, 28(11), 2411-2414.
17. Raskop, M., Seubert, A., Grimm, A., Ion Exchange Material, Ion Exchange column and method of preparation. EP Patent 1,842,592., 2007.
18. Sonnenschein, L., Seubert, A., Synthesis of a series of monomeric styrene sulfobetaine precursors. Tetrahedron Letters2011, 52(10), 1101-1104.
19. http://chem2.sis.nlm.nih.gov/chemidplus/name, log POW values by US National Library of Medicine. 2016.
20. Haddad, P.R., Jackson, P.E., Ion chromatography: principles and applications. Elsevier Amsterdam. 1990.
21. Predicted chemicalize.org beta by ChemAxon (http://www.chemicalize.org/).

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