Sys Rev Pharm 2020; 11(2): 388 – 394
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Development of ZIC-HILIC Methods Using Ultraviolet Detection for determining 2-deoxyuridine in Human Serum

Ashraf Saad Rasheed[*1], Farah A. Rashid[2]
[1]Department of Chemistry, College of Sciences, University of Baghdad, Al-Jadriya campus, 10071 Baghdad, Iraq.
[2]Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq
[*]E-mail: Ashraf_analytical@yahoo.com

Article History: *Submitted: 20.12.2019* *Revised: 11.01.2020* *Accepted: 13.02.2020*

ABSTRACT
The work has been carried out the hydrophilic chromatographic separation of 2-deoxyuridine. Two handmade ZIC-HILIC columns with eluent (A-10%, acetate buffer 5 mM (pH 4.75); B-90%, of ACN) were used. The separation took place at 9 min with a flow rate of 0.75 mL/min. In application to the serum from humans, the quantification of 2-deoxyuridine was performed. The retention mechanism of the columns was studied in view of the use of 2-deoxyuridine for hydrophilic and anionic interactions. Such techniques are a valuable alternative to conventional 2-deoxyuridine separation methods. The LOD and LOQ were respectively 0.03 and 0.01 µg.mL^{-1} for two methods. The validation parameters produced recovery rates from 96.95±0.83 to 97.39±0.63 with the precision of 1.44 ± 0.16 and 1.26 ± 0.25%, respectively, as a relative standard deviation.

Keywords: 2-deoxyuridine, ZIC-HILIC, anion exchange interaction, human serum

Correspondence:
Ashraf Saad Rasheed
Department of Chemistry, College of Sciences, University of Baghdad
Iraq
E-mail: Ashraf_analytical@yahoo.com

DOI: 10.5530/srp.2020.2.57

INTRODUCTION
In the past years, Retention and separation in the suitable chromatographic mode of the polar and hydrophilic molecules has been developed as hydrophilic-liquid chromatography (HILIC). It was adopted that the principal retention mechanism is the division between the column's aqueous layer and the eluent's organic portion.. The presence of the secondary interactions (for example, ion-exchange, hydrogen bonding and the dipole-dipole) may, however, play a significant role, leading to selectivity changes. Because HILIC mode is becoming increasingly popular in polar and ionic analysis, increasingly HILIC columns are commercially available [1]. The new materials are continuously produced and tested as columns under HILIC circumstances in various types of polar functional groups (including sulfobetaine, phosphocholine, carboxybetaine, cyano, amide, cyano, diol) [1-4]. The basic components of every cell containing different nucleic acids are metabolite compounds. In recent decades their clinical interest in bioanalysis has expanded considerably, thus nucleosides are investigated and excluded with or without changes in the urinary tract as potential biomarkers of the oncological processes [5-7]. In addition to biology, in other avenues of investigation nuclear bases, nucleosides and nucleotides are also essential. Nutrients of particular importance in foods during or after rapidly growing periods are monophosphate nucleotides and therefore the additional contribution that these nutrients make to neonatal feed is important [8, 9]. Successful implementation of HILIC technology has been carried out analyzing different analytes [2, 10-14]. The class of organic compounds known as pyrimidine 2'-deoxyribonucleoside is deoxyuridine (Figure1). 2'-deoxyribonucleosides are pyrimidine-related compounds which, at position 2, have no hydroxyl group. In all living organisms, deoxyuridine exists and extends from humans to bacteria. The deoxyuridine is involved in a range of enzyme reactions in human beings [15].

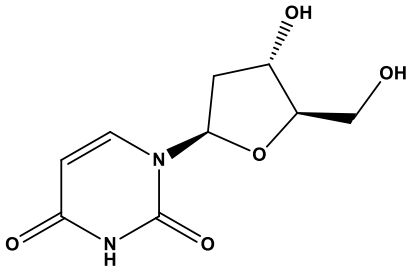

Figure 1: Structure of 2-deoxyuridine.

For different samples, multiple methods are available to isolate and estimate nucleosides [16-21]. The purpose of this study was to describe the behavior of 2-deoxyuridine ZIC1 and ZIC5 in homemade ZIC-HILIC columns. 2-deoxyuridine retention was systematically studied to detect their potential relevance in serum samples and to optimize the circumstances for their separation in gradient elution. Moreover, the influence on 2-deoxyuridine retention has not yet been studied in the chain length of the ZIC-HILIC columns.

EXPERIMENTAL
2.1 chemicals
Sigma-Aldrich bought 2-deoxyuridine (HPLC- 98.5%). The HPLC acetonitrile grade was bought to Sigma-Aldrich. Fluka has been obtained with sodium acetate. BDH was bought with acetic acid. Capacities for ZIC1 and ZIC5 are available respectively at 432 and 488 µeq g^{-1} [22].

2.2 Chromatographic circumstances and instrumentation
With its gradient pump L-6200 and the visible L-4200 UV, the Merck Hitachi HPLC System has a 20 µL injection loop. My chromatography and analysis were supervised by N2000 Data Workstation tools. Ultraviolet areas at a wavelength of 254 nm were used for the detection of 2-deoxyuridine. The PS / DVB grafted monomer sulfobetaine (100 mm x 4 mm I.D), as well as the column PEEK, were designed to separate 2-deoxyuridine using ZIC1 and ZIC5 columns [2, 3, 11, 23]. Raskop et al. [24] have identified the systemic process of the grafting reaction.

RESULTS AND DISCUSSION

3.1 The optimum separation of 2-deoxyuridine

The separation of the HILIC-mode, 2-deoxyuridine was tested by a mobile phase process in the ZIC1 and ZIC5 columns. Figures 2 and 3 display the chromatograms. A sodium acetate of 5 mM (pH 4.75) and 90% of ACN was used to produce chromatograms.

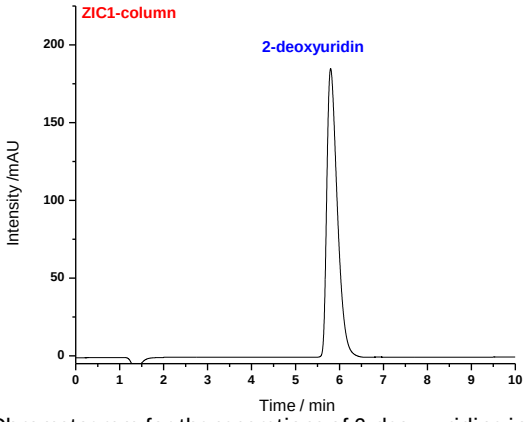

Figure 2: Chromatogram for the separations of 2-deoxyuridine in ZIC1-column.

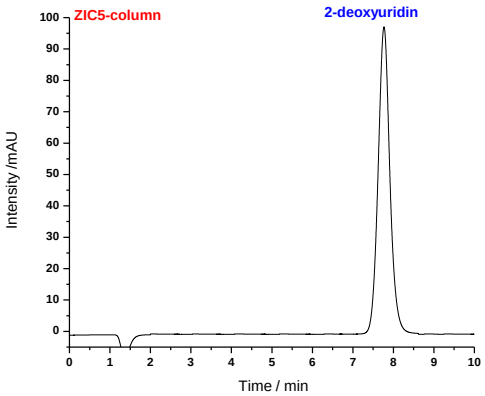

Figure 3: Chromatogram for the separations of 2-deoxyuridine in ZIC5-column.

As shown in the ZIC5 column, in Figures 2 and 3 the interaction between the 2-deoxyuridine and the column increases the duration of retention of 2-deoxyuridine. It is due to the increasing methyl group in the ZIC column between charges. In mobile phase compounds, the systemic ACN variation is between 50% and 95%. The eluent buffer concentration of 5 to 25 mM at pH levels from 3 to 4.75 ensures the separative characteristics and separation mechanism of both columns are evaluated.

3.2 Influence of 2-deoxyuridine retention on ACN content

Increased nucleoside retention by the increasing ACN content of ZIC-HILIC mode Moreover, hydrophilic (HILIC) behaviors are indicating nucleosides with lower mobile phase water content. This behavior difference is due to the hydrophilicity of the nucleosides. The HILIC behavior (Figures 4 and 5) is shown in ZIC1 and ZIC5 columns. The explanation is because of 2-deoxyuridine $logP_{Octanol/Water}$ (-1.14) [25, 26].

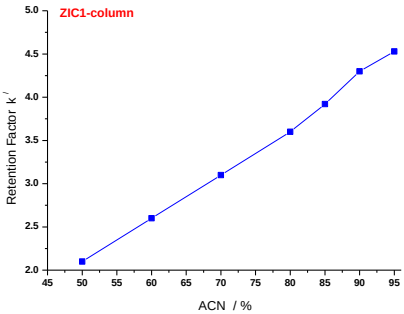

Figure 4: ACN content influence on 2-deoxyuridin retention in ZIC1-column.

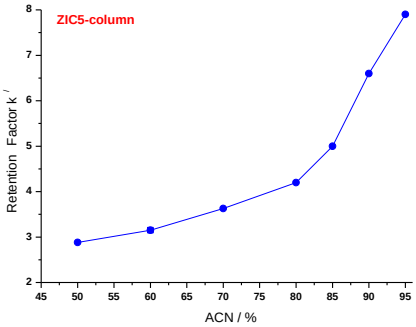

Figure 5: ACN content influence on 2-deoxyuridine retention in ZIC5-column.

3.3 Buffer concentration influence on retention of 2-deoxyuridine

Higher buffer concentrations in the mobile phase were usually increased for HILIC retention which caused ion pairs to be deactivated. Therefore, when ACN is active, a linearization of functional groups is enhanced [3]. The retention of nucleosides has increased or decreased in HILIC columns with an increase in buffer amount [16, 27]. The 2-deoxyuridine retention factor is a decrease from 5 to 25 mM and a pH of 4.75 and up to ACN 90 percent as shown in Figures 6 and 7. In the standard columns of ion exchange, this slope [28] is similar to slopes obtain in Figures 6 and 7 (0.3458 and 0.3457). The image of 2-deoxyuridine is different as the buffer increases due to two retention factors. The core column and 2-deoxyuridine. Therefore, 2-deoxyuridine the value of the isoelectric point (2.82) remains anionic. The interaction of 2-deoxyuridine with HILIC relied on the anion exchange.

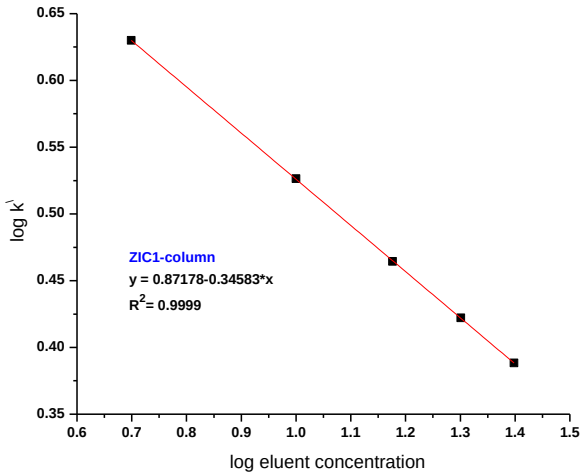

Figure 6: Concentration influence of eluent in ZIC1-column on 2-deoxyuridine retention.

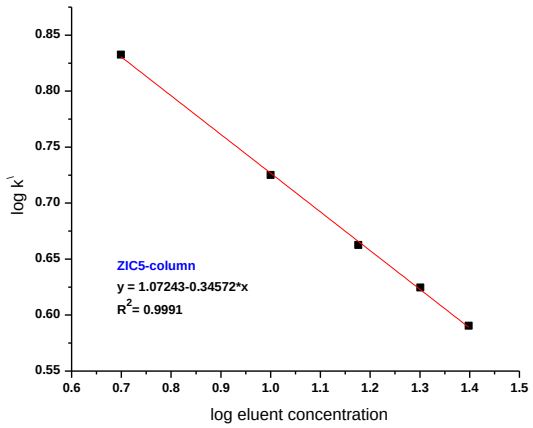

Figure 7: Concentration influence of eluent in ZIC5-column on 2-deoxyuridine retention.

3.4 Eluent pH influence on 2-deoxyuridine retention

The eluent pH must vary in order to complete the concept of 2-deoxyuridine separation. The retention of 2-deoxyuridine increased from 3 to 4.75 with levels of sodium acetate held at 5 m M and ACN of 90%, as shown in figures 8 and 9. Due to the amino group deprotonation of2-deoxyuridine, 2-deoxyuridine preservation is increased during the ZIC1 and ZIC5 columns.

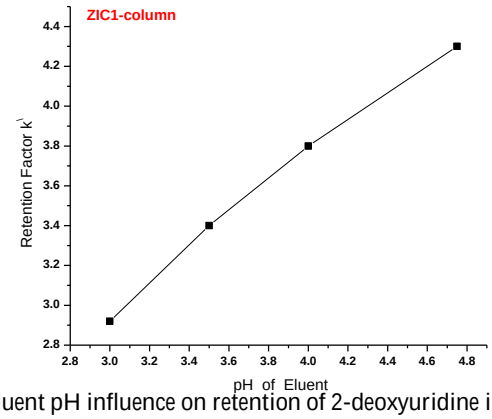

Figure 8: Eluent pH influence on retention of 2-deoxyuridine in column ZIC1.

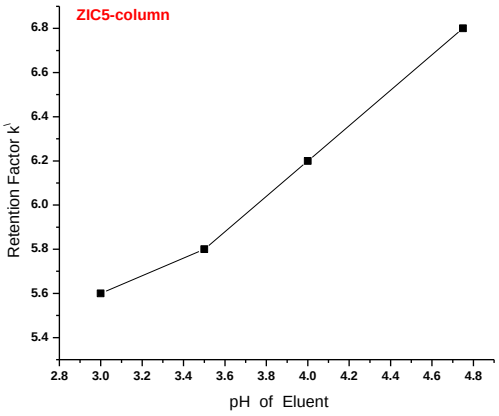

Figure 9: Eluent pH influence on retention of 2-deoxyuridine in column ZIC5.

3.5 Method validation

In the conditions of the study mentioned, a linear relation between the 2-deoxyuridine concentrations and the peak area of 254 nm has been achieved. This linearity of 0.1–5 µg.ml^{-1} was preserved for 2-deoxyuridine in two methods, which can be seen in Figure 10. The related calibration graphs and statistical findings in Table 1 were used for the comprehensive analysis under the HILIC circumstances of 2-deoxyuridine. In terms of precision, recovery, the reliability of both methods has been studied in the HILIC circumstances. The accuracy and precision of three concentrations were evaluated (Table 2).

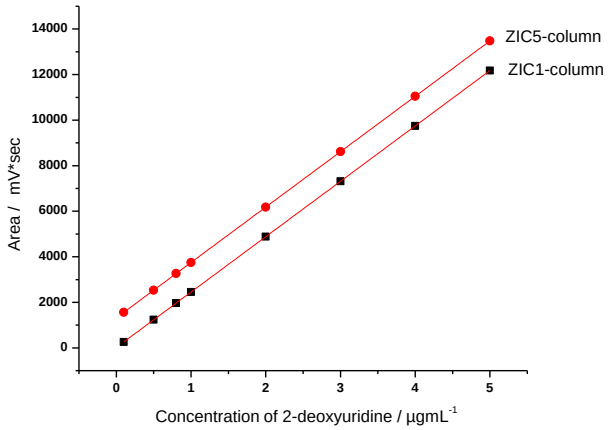

Figure 10: Curves of calibration using ZIC1 and ZIC5 columns for determination 2-deoxyuridine.

Table 1: Statistical findings for both strategies (ZIC1 and ZIC5) for the recoveries.

Parameter	ZIC1 method	ZIC5 method
Linearity (µg.ml^{-1})	0.1-5	0.1-5
Regressiona equation	y= 18+ 2432*x	y = 1318+ 3532*x
r^2	0.9995	0.9991
LOD (µg.mL^{-1})	0.03	0.01
LOQ (µg.mL^{-1})	0.105	0.035

Table 2: The accuracy and precision of two methods.

	Intraday-analysis n=5				Interday-analysis n=5			
ZIC1 method								
dU Taken µg.mL^{-1}	dU Found µg.mL^{-1}	%Rec.	% $E_{rel.}$	%RSD	dU Found µg.mL^{-1}	%Rec.	% $E_{rel.}$	%RSD
1.00	0.981	98.10	-1.90	1.66	0.983	98.30	- 1.70	1.73
2.00	1.923	96.15	-3.85	1.42	1.955	97.75	-2.25	1.48
3.00	2.898	96.60	-3.4	1.26	2.915	97.16	-2.84	1.33
ZIC5 method								
1.00	0.982	98.20	- 1.80	1.51	0.984	98.40	- 1.60	1.56
2.00	1.933	96.65	-3.35	1.36	1.936	96.80	-3.2	1.48
3.00	2.920	97.33	-2.67	0.92	2.910	97.00	-3.00	1.10

3.5.2 Use in human serum spiked samples of the HILIC method detection study to evaluate 2-deoxyuridine.

Two methods were used to measure in-vitro 2-deoxyuridine in a spiked human serum with two levels, according to the findings from Table 3. The proposed methods were successfully applied. The results obtained by a comparative method [27] were contrasted with those for the assessment of the competency and efficiency of the ZIC1and ZIC5 methodologies. Test results (table 4) and variance ratios (95%) were used for statistical analyses. The measured values of t and F do not go beyond the theoretical value, which means the exact determination of 2-deoxyuridine on the human serum sample does not vary considerably in both methods.

Table 3: Two strategies for 2-deoxyuridine in human serum.

2-deoxyuridin Taken µg.mL^{-1}	2-deoxyuridin Found µg.mL^{-1}	%Rec.	%$E_{rel.}$	%RSD n=5
ZIC1 method				
1.00	0.979	97.90	-2.10	1.53
2.00	1.930	96.50	-3.50	1.30
3.00	2.893	96.43	-3.57	1.11
ZIC5 method				
1.00	0.963	96.30	-3.70	1.71
2.00	1.941	97.05	-2.95	1.48
3.00	2.885	96.16	-3.84	0.98

Table 4: Using t-tests and F-tests to equate the comparative approach to the two alternative HILIC approaches [27].

Name of nucleo-side	ZIC1 method	ZIC5 method	Comparison [27] method	t-Test (the-or.)	F-Test (the-or.)
2-deoxyuridin	97.90	96.30	96.58	0.7046* (2.7764)	2.1116* (19.00)
	96.50	97.05	96.21	0.6609** (2.7764)	0.7033** (19.00)
	96.43	96.16	97.33		

*For ZIC1 approach

**For ZIC5 approach

CONCLUSION

Gradient elution with sodium acetate buffer-ACN eluent on two ZIC-HILIC homemade columns separated 2-deoxyuridine in under 9 min. Both methods are faster. They are more sensitive, efficient and separate better than existing methods. It satisfies the requirements for linearity, the limit of detection, the limit of quantitation, repeatability, precision, and recovery. Such methods are successful in the determination in human serum samples of 2-deoxyuridine. This article deals with HILIC methods for the assessment of 2-deoxyuridine in human serum samples. A flexible separate method that offers HILIC columns with one or five methylene groups between charged groups at least two different modes under different circumstances. The interaction between 2-deoxyuridine and ZIC5 was found to be longer. This can be traced to the geometric orientation of the column ZIC5. The evidence seems to indicate that hydrophilic and anion exchange interactions are the retention mechanism. The methods developed successfully have been used in human serum samples.

REFERENCES

1. D. García-Gómez, E. Rodríguez-Gonzalo, and R. Carabias-Martínez, "Stationary phases for separation of nucleosides and nucleotides by hydrophilic interaction liquid chromatography," *TrAC Trends in Analytical Chemistry,* vol. 47, pp. 111-128, 2013.
2. A. S Rasheed and A. Seubert, "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 Chromatography,* vol. 3, pp. 4-11, 2016.
3. A. S. Rasheed, B. A. Al-Phalahy, and A. Seubert, "Studies on Behaviors of Interactions Between New Polymer-based ZIC-HILIC Stationary Phases and Carboxylic Acids," *Journal of chromatographic science,* vol. 55, pp. 52-59, 2016.
4. E. P. Nesterenko, P. N. Nesterenko, and B. Paull, "Zwitterionic ion-exchangers in ion chromatography: A review of recent developments," *Analytica chimica acta,* vol. 652, pp. 3-21, 2009.
5. A. Frickenschmidt, H. Fröhlich, D. Bullinger, A. Zell, S. Laufer, C. H. Gleiter, H. Liebich, and B. Kammerer, "Metabonomics in cancer diagnosis: mass spectrometry-based profiling of urinary nucleosides from breast cancer patients," *Biomarkers,* vol. 13, pp. 435-449, 2008.
6. G. CM, "Mackey JR. Dumontet C," *Lancet Oncol,* vol. 3, p. 415, 2002.
7. A. Seidel, S. Brunner, P. Seidel, G. Fritz, and O. Herbarth, "Modified nucleosides: an accurate tumour marker for clinical diagnosis of cancer, early detection and therapy control," *British journal of cancer,* vol. 94, pp. 1726-1733, 2006.
8. J. D. Carver, "Advances in nutritional modifications of infant formulas," *The American journal of clinical nutrition,* vol. 77, pp. 1550S-1554S, 2003.
9. E. Schlimme, D. Martin, and H. Meisel, "Nucleosides and nucleotides: natural bioactive substances in milk and colostrum," *British Journal of Nutrition,* vol. 84, pp. 59-68, 2000.
10. A. S. Rasheed, B. A. Al-Phalahy, and A. Seubert, "Studies on Behaviors of Interactions Between New Polymer-based ZIC-HILIC Stationary Phases and Carboxylic Acids," *Journal of chromatographic science,* vol. 55, pp. 52-59, 2017.
11. B. A. Al-Phalahy, Y. H. Muhamad, and A. S. Rasheed, "Zwitterionic Ion Chromatography of Dansyl Amino Acids with 4-Vinylbenzyl Dimethyl Ammonio Pentanesulfonate as Stationary Phase," *Asian Journal of Chemistry,* vol. 28, p. 2411, 2016.
12. M. A. Abbas and A. S. Rasheed, "Study on the retention behavior of famotidine in hydrophilic interaction liquid chromatography."
13. M. A. Abbas and A. S. Rasheed, "Famotidine determination in pure and pharmaceutical formulations by zwitterionic chromatography-hydrophilic interac-tion liquid chromatography."
14. J. K. Palmer, "A versatile system for sugar analysis via liquid chromatography," *Analytical letters,* vol. 8, pp. 215-224, 1975.
15. D. S. Wishart, Y. D. Feunang, A. Marcu, A. C. Guo, K. Liang, R. Vázquez-Fresno, T. Sajed, D. Johnson, C. Li, and N. Karu, "HMDB 4.0: the human metabolome database for 2018," *Nucleic acids research,* vol. 46, pp. D608-D617, 2018.
16. G. Marrubini, B. E. C. Mendoza, and G. Massolini, "Separation of purine and pyrimidine bases and nucleosides by hydrophilic interaction chromatography," *Journal of separation science,* vol. 33, pp. 803-816, 2010.
17. G. Zhou, H. Pang, Y. Tang, X. Yao, Y. Ding, S. Zhu, S. Guo, D. Qian, J. Shen, and Y. Qian, "Hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HILIC-UPLC–TQ-MS/MS) in multiple-reaction monitoring (MRM) for the determination of nucleobases and nucleosides in ginkgo seeds," *Food chemistry,* vol. 150, pp. 260-266, 2014.
18. M. Mateos-Vivas, E. Rodríguez-Gonzalo, J. Domínguez-Álvarez, D. García-Gómez, and R. Carabias-Martínez, "Determination of nucleosides and nucleotides in baby foods by hydrophilic interaction chromatography coupled to tandem mass spectrometry in the presence of hydrophilic ion-pairing reagents," *Food chemistry,* vol. 211, pp. 827-835, 2016.
19. E. Rodríguez-Gonzalo, D. García-Gómez, and R. Carabias-Martínez, "Study of retention behaviour and mass spectrometry compatibility in zwitterionic hydrophilic interaction chromatography for the separation of modified nucleosides and nucleobases," *Journal of Chromatography A,* vol. 1218, pp. 3994-4001, 2011.
20. P. Chen, W. Li, Q. Li, Y. Wang, Z. Li, Y. Ni, and K. Koike, "Identification and quantification of nucleosides and nucleobases in Geosaurus and Leech by hydrophilic-interaction chromatography," *Talanta,* vol. 85, pp. 1634-1641, 2011.
21. S. Guo, J.-a. Duan, D. Qian, H. Wang, Y. Tang, Y. Qian, D. Wu, S. Su, and E. Shang, "Hydrophilic

interaction ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry for determination of nucleotides, nucleosides and nucleobases in Ziziphus plants," *Journal of Chromatography A,* vol. 1301, pp. 147-155, 2013.

22. M. A. Abbas and A. S. Rasheed, "Retention characteristic of ranitidine hydrochloride on new polymer-based in zwitter ion chromatography-hydrophilic interaction chromatography stationary phases," *J. Chem. Soc. Pak,* vol. 40, p. 89, 2018.
23. A. Seubert and A. Saad Rasheed, "Separation of Metal-Trifluoperazine Hydrochloride Complexes Using Zwitterionic Ion Chromatography (ZIC) Coupled Online with ICP-AES," *Current Pharmaceutical Analysis,* vol. 13, pp. 328-333, 2017.
24. M. Raskop, A. Seubert, and A. Grimm, " Ion Exchange Material, Ion Exchange column and method of preparation.," *EP Patent 1,842,592,* 2007.
25. "URL:https://ilab.acdlabs.com/iLab2/. E-mail: ashraf_analytical@yahoo.com."
26. "Predicted- chemicalize.org beta by ChemAxon (http://www.chemicalize.org/)."
27. Y. Chen, W. Bicker, J. Wu, M. Xie, and W. Lindner, "Simultaneous determination of 16 nucleosides and nucleobases by hydrophilic interaction chromatography and its application to the quality evaluation of Ganoderma," *Journal of agricultural and food chemistry,* vol. 60, pp. 4243-4252, 2012.
28. P. R. Haddad and P. E. Jackson, *Ion chromatography* vol. 46: Elsevier, 1990.