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Zwitterionic Ion Chromatography Coupled with Ultraviolet Detection for the Quantification of 2-Deoxyguanosine in Human Serum

Yaqout Abd Al-Hakeem Hamed[1], Ashraf Saad Rasheed[*1]
[1]Department of Chemistry, College of Sciences, University of Baghdad, Al-Jadriya campus, 10071 Baghdad, Iraq.
[*]E-mails: Ashraf_analytical@yahoo.com

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ABSTRACT
A simple ZIC-HILIC-UV technique was developed and applied for the determination of 2-deoxyguanosine in the human serum. The separation took place in the ZIC1 and ZIC5 self-designed stationary phases, in gradient elution mode with a buffer for acetonitrile acetate and 254 nm UV detection. The 2-deoxyguanosine conduct with varying acetate buffer, ACN and pH values has been tested and the results have established the hydrophilicity of 2-deoxyguanosine. The mechanism of separation is based on the hydrophilic and ion exchange partitioning of the2-deoxyguanosine. We have defined and mentioned the current effect of both ZIC1 and ZIC5 stationary stationary phenomena on chromatographic conditions (sodium acetate buffer concentration, ACN and pH). All existing methods are a useful alternative to the current 2-deoxyguanosine separation methods.

Keywords: 2-deoxyguanosine, cation exchange, nucleoside, HILIC stationary phase, hydrophilic interaction

Correspondence:
Ashraf Saad Rasheed
Department of Chemistry, College of Sciences, University of Baghdad, Al – Jadriya Campus, 10071, Baghdad, Iraq
E-mail: Ashraf_analytical@yahoo.com

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INTRODUCTION
In all living organisms, nucleosides are the most essential nitrogen molecules. For although purine and pyrimidine nucleosides, nuclear acid is a metabolite that is part of the biological energy processes and is involved among syntheses such as polysaccharides, phospholipids and macromolecular glycosides [1]. It is also possible to add nucleosides to the body and exogenously absorb by feeding [2]. For many biological processes, nucleotides are essential. DNA (Deoxyribonucleic Acid) and RNA (ribonucleic acid) genetic information for storing, transmitting, and expressing DNA in all the natural ingredients of humans, bacteria and plant cells [3]. The class of organic compounds known as 2'deoxyribonucleoside purines is deoxyguanosine, also known as dG (Figure1). Purine 2'-deoxyribonucleosides consists of a purine associated with a ribose, which at position 2 has no hydroxyl group. When a group of phosphates is set to 5', it becomes monophosphate deoxyguanosine. In all living species, Deoxyguanosine occurs, from bacteria to humans. Deoxyguanosine is involved in a number of enzymatic reactions in human beings. In general, the enzyme cytosolic purine 5'-nucleotidase may be used to synthesize deoxyguanosine from 2'-deoxyguanosine 5'-monophosphate. Deoxyguanosine can also be converted by its interaction with the deoxyguanosine kinase enzyme into 2'-deoxyguanosine 5''-monophosphate [4].

Alpert coined the term HILIC (hydrophilic interaction chromatography) in 1990 [5], and many studies have shown the efficient technique of hydrophilic interaction between liquid chromatography and various polar compounds [5]. HILIC's approach to several previously intimidating separation problems is currently strongly attracted. The analysis of pharmaceuticals [6-11], dansyl amino acids [12], inorganic anions [13], carboxylic acid [14], sugar [15] and saccharides [16] by the successful implementation of HILIC-technology has been achieved.

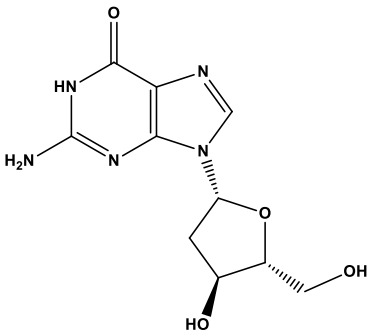

Figure 1: Structure of 2-deoxyguanosine (dG)

Many approaches for nuclear separation and evaluation are available for various applications [17-22]. This study shows the behavior of 2-deoxyguanosine, ZIC1, and ZIC5, with ZIC-HILIC stationary phases. This research represented the actions of 2-deoxyguanosine with the ZIC-HILIC exchangers, namely ZIC1 and ZIC5. The retention of 2-deoxyguanosine was methodically checked in order to detect the applicability potential of these columns in serum samples and optimal conditions were used for gradient elution separation. Therefore, impacts of ZIC-HILIC stationary phases chain lengths have not been examined on 2-deoxyguanosine retention behavior.

EXPERIMENTAL
Instrumentation and chromatographic condition
The Merck Hitachi HPLC System has a 20 µL injection loop with the gradient pump L-6200 and the visible L-4200 ultraviolet. The N2000 Data Workstation software was used to monitor my chromatography and analysis. For the detection of 2-deoxyguanosine, the ultraviolet region was used at a wavelength of 254 nm. ZIC1 and ZIC5 were developed on the PS / DVB, by means of a grafted sulfobetaine monomer (100 mm x 4 mm I.D) and PEEK column, for the 2-deoxyguanosine separation [6, 12-14]. The systematic

cycle of grafting reaction has been established by Raskop et al. [23].

chemicals

2-deoxyguanosine (HPLC) purchased by Sigma-Aldrich 99%. Acetic acid was purchased from BDH. Sodium acetate was obtained for Fluka. Sigma-Aldrich was awarded the HPLC acetonitrile grade. For ZIC1 and ZIC5, the capacity is available in 432 and 488 μeq g^{-1} [7] respectively.

RESULTS AND DISCUSSION

The optimum separation of dG

In the stationary phases of ZIC1, ZIC5, the separation of HILIC-mode dG was tested by a mobile phase protocol. In chromatograms (Figures 2 and 3), 5 mM sodium acetate (pH 3) and 80% ACN were obtained.

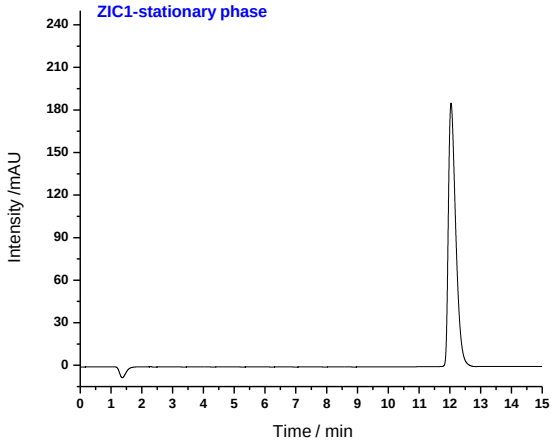

Figure 2: Chromatogram for the separations of dG in the ZIC1-stationary phase.

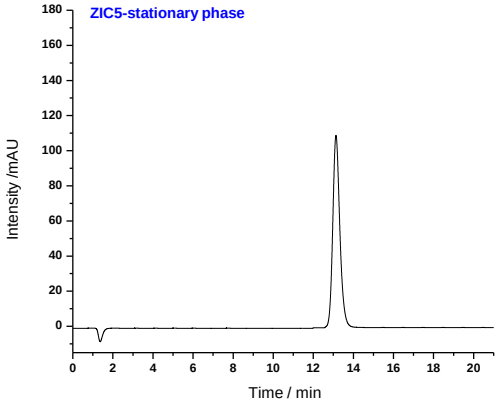

Figure 3: Chromatogram for the separations of dG in ZIC5-stationary phase

The interaction of the dG and the stationary phase increases the chain length and the retention time of the dG, as demonstrated at the stationary phase ZIC5, in figures 2 and 3. This is because between charges the methyl group is through in the ZIC stationary phase. In the mobile phase composites, the systemic variability of ACN ranges between 60% and 95%. The eluent concentration from 5 to 25 mM at the pH levels from 3 to 5.5 ensures the separation characteristics of each stationary phase, and thus tests the separation mechanism.

Effect of dG retention on ACN content

Improved interaction of nucleoside through the increase of ACN content in ZIC-HILIC mode. In addition, hydrophilic activities (HILIC) suggest a lower content of nucleosides in the Mobile phase water. The hydrophilicity of the dG is responsible for this conduct difference. The dG exhibits HILIC behavior in the ZIC1 and ZIC5 stationary phases (Figure 4 and Figure 5). This is due to its $logP_{ow}$ of dG (-1.8) [24, 25].

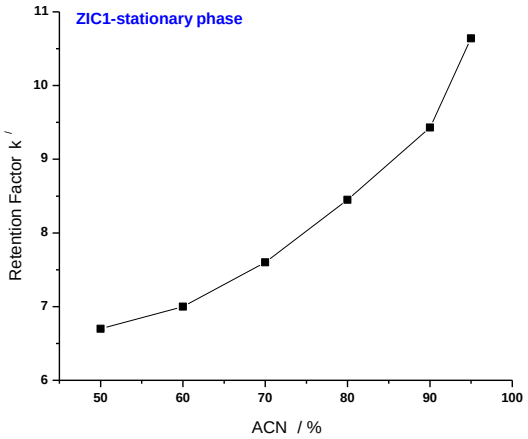

Figure 4: ACN content impact on dG retention in the ZIC1-stationary phase.

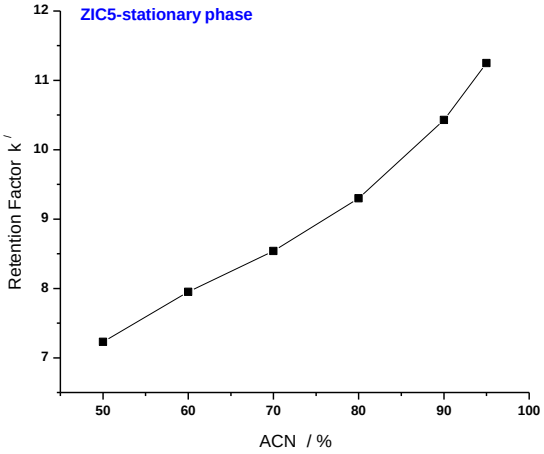

Figure 5: ACN content impact on dG retention in the ZIC5-stationary phase.

Eluent impact on retention of dG

For ZIC-HILIC interactions, the eluent levels were normally increased and intramolecular ion pairs suppression. This increases the linearization of functional groups if ACN occurs [12]. The retention of nucleosides in HILIC exchangers is reduced or increased with a higher buffer degree [17, 26]. The cations exchange [12] illustrates this. As shown in figures 6 and 7, the dG k\ decreases with the NaOAc buffer increasing from 5 to 25 mM, pH 3 at 80% ACN. These slopes are obtained by Figures 6 and 7 (0.3452 and 0.3500) are similar to regular ion-exchange stationary phases [27]. The value of dG isoelectric points (5.13), which therefore remains cationic form, and then depends on the cation exchange of the interaction between dG and ZIC-HILIC stationary phase.

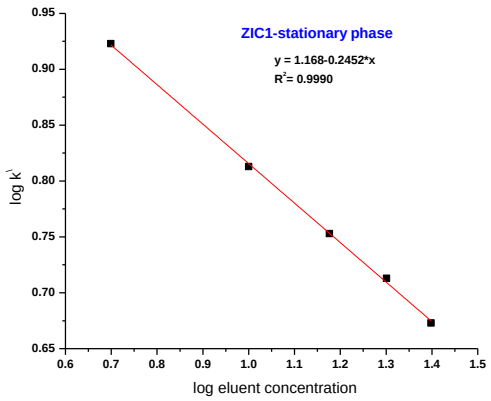

Figure 6: Eluent concentration impact on dG retention in ZIC5-stationary phase

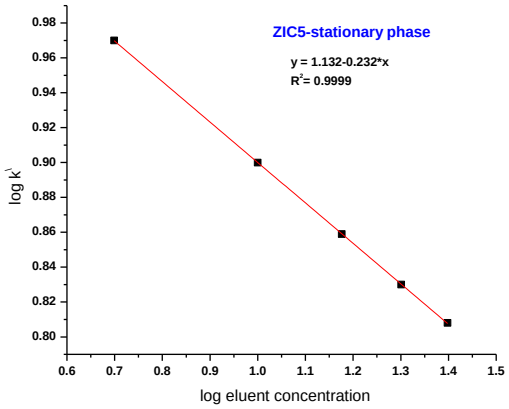

Figure 7: Eluent concentration impact on dG retention in the ZIC5-stationary phase.

Eluent pH effect on dG retention

The eluent pH has been raised from 3 to 5.5, the sodium acetate level retained at 5 mM and 80 percent of ACN, and hence the dG retention factor has been decreased (Figures 8 and 9). The eluent must have a pH variable for the completion of the dG separation principle. Due to the deprotonation of the amino group in dG, the interaction of dG is increased in stationary ZIC1 and ZIC5 phases

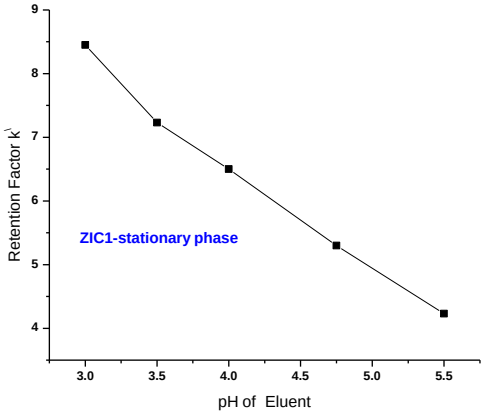

Figure 8: Eluent pH impact dG retention in the ZIC1-stationary phase.

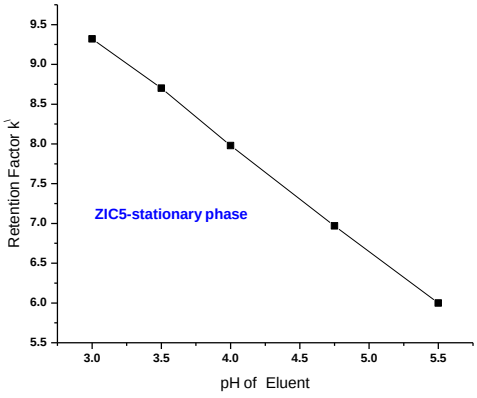

Figure 9: Eluent pH impact dG retention in the ZIC5-stationary phase.

Validation

The linearity (0.01-15 $\mu g\ mL^{-1}$) of two ZIC1 and ZIC5 stationary phases with dG can be observed, as shown in Figure 10.

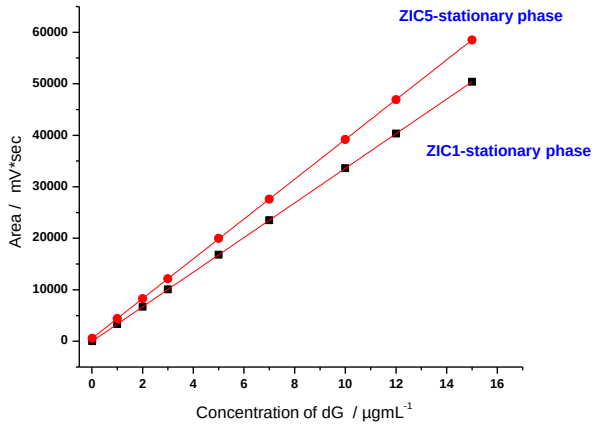

Figure 10: Calibration graphs for dG using ZIC1 and ZIC5 stationary phases.

For extensive testing of dG under HILIC conditions, correct calibration graphs and statistical data were used in Table 1. The exactness of recovery and %RSD was calculated on the interday and intraday analysis. The very low defaults and high recovery values (Table 2) show a successful strategy.

Table 1: LOD, LOQ and linear regression data of the dG.

Parameter	ZIC1 method	ZIC5 method
Linearity (µg.ml^{-1})	0.03-10	0.03-10
Regression equation	y= -17.04+ 3360.63*x	y = 557.24+ 3864.24*x
r^2	0.9995	0.9992
LOD (µg.mL^{-1})	0.008	0.006
LOQ (µg.mL^{-1})	0.028	0.021

Table 2: Recovery of the existing methodologies on interday and intraday analysis

	Intraday-analysis n=5				Interday-analysis n=5			
ZIC1 method								
dG Taken µg.mL^{-1}	dG Found µg.mL^{-1}	%Rec.	% $E_{rel.}$	%RSD	dG Found µg.mL^{-1}	%Rec.	% $E_{rel.}$	%RSD
3.00	2.989	99.63	-0.37	1.23	2.983	99.33	-0.67	1.61
5.00	4.932	98.64	-1.36	0.98	4.988	99.76	-0.24	1.46
8.00	8.032	100.40	0.40	0.77	8.020	100.25	0.25	1.37
ZIC5 method								
3.00	2.985	99.50	- 0.50	1.58	3.020	100.66	0.66	1.73
5.00	5.023	100.46	0.46	1.23	4.983	99.66	-0.34	1.35
8.00	7.995	99.93	- 0.07	0.93	7.990	99.87	-0.13	1.18

dG detection in human serum spiked samples

Taking two techniques for the measurement of in-vitro dG in a spiked serum with 2 concentrations, the proposed strategies were successful, Table 3 reports the findings.

Table 3: Two techniques for human serum dG assessment have been proposed.

dG Taken µg.mL^{-1}	dG Found µg.mL^{-1}	%Rec.	%$E_{rel.}$	%RSD n=5
ZIC1 method				
3.00	2.975	99.16	-0.84	1.72

5.00	4.920	98.40	-1.60	1.67
8.00	7.893	98.66	-1.34	1.39
ZIC5 method				
3.00	2.983	99.43	-0.57	1.65
5.00	4.932	98.64	-1.36	1.52
8.00	7.850	98.12	-1.88	1.33

The findings of the comparative method [28] were compared with the results obtained in evaluating the competence and efficiency of the ZIC1/ZIC5 methods. Test results (Table 4) in variances ratios (95 percent) for statistical analyses were used. The calculated t and F values are not above the theoretical value so that the dG accuracy in the human serum sample in both methods cannot be substantially differentiated.

Table 4: Comparison of the proposed ZIC1 and ZIC5 methods with the dG test method [28] for an examination of t- and F-statistical tests.

Name of nucleoside	ZIC1 method	ZIC5 method	Comparison [28] method	t-Test (theor.)	F-Test (theor.)
2-deoxyguanosine	99.16	99.43	98.55	0.7093* (2.7764)	0.6337* (19.00)
	98.40	98.64	98.66	0.7619** (2.7764)	1.848** (19.00)
	98.66	98.12	99.44		

*For ZIC1 method
**For ZIC5 method

CONCLUSION

This paper explores the application of HILIC techniques in human serum samples for the evaluation of 2-deoxyguanosine. This flexible separation is useful because HILIC traders with one and five methylene groups have at least two different holding modes between charged groups under different conditions. This can be due to the geometrical orientation of the ZIC5 exchanger. The data indicate that both hydrophilic and cation behaviors are the retention mechanism. The strategies established have been successfully implemented in human serum samples.

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