ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.SP2.050

Research article

Development and validation of hydrophilic interaction chromatography for determination of deoxyadenosine in human serum

ASHRAF SAAD RASHEED*[1], AMEEN WALEED QASSIM[2], SALSAL KAMAL ABDULRAHMAN[2]
[1]Department of Chemistry, College of Science, University of Baghdad, Al-Jadriya campus, 10071 Baghdad, Iraq.
[2]Baghdad College of Medical Science/ MOHESR/Iraq
Corresponding Author:
Email: Ashraf_analytical@yahoo.com[1]
Received: 16.07.20, Revised: 21.08.20, Accepted: 08.09.20

ABSTRACT
For deoxyadenosine determination, a straightforward ZIC-HILIC-UV method was developed and used to human serum.The separation was carried out by gradient elution mode with acetonitrile acetate buffer and UV detection of 254 nm, on ZIC2 and ZIC5 self-constructed columns. The deoxyadenosine behavior with various acetate buffer, ACN proportion, and pH values was examined and the findings confirmed the deoxyadenosine hydrophilicity. The separation mechanism is premised on the partitioning of the deoxyadenosine in the hydro-philic and ion exchange. We mentioned and explained the present impact of chromatographic circumstances (sodium acetate buffer concentration, ACN, and the pH) for both ZIC1 and ZIC5 columns. The two existing methods are a useful alternative to current separation methods for deoxyadenosine.

Keywords: deoxyadenosine, ion exchange, nucleoside, ZIC- HILIC, hydrophilic interaction

INTRODUCTION

The derivative of nucleoside adenosine is deoxyadenosine (Figure1). Adenine is a deoxyribose movement through an N9-glycoside bond. It is adenine. The absence of oxygen in the 3-inch position of its Ribose Ring varies from Ri-bose. Deoxyadenosine is an essential DNA com-ponent. Deoxyadensoine can function as an immunotoxin and a metabotoxin if it is present at sufficiently high levels. Impairment, failure or loss of immune cells is caused by the immunotoxins. A metabotoxin is a metabolite endogenous that in-duces chronically high adverse health effects [1]. Nucleosides are the most essential nitrogen molecules in all living organisms. Nuclear acid, both metabolite involved in biological energy proce-dures and syntheses, such as polysaccharides, phospholipids, and glycosides of macromolécules, are essential for alt-hough pu-rine and pyrimidine nucleosides [2]. Nucleosides can also be incorporated into the body and used exogenously by diet [3]. Numerous biological functions require nucleotides. Nucleic acids are the natural precursors for the storage, transport, and expression of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) genetic information in all animals, bacteria, and plant cells [4].

HILIC (hydrophilic interaction chromatography) was coined by Alpert in 1990 [5]. Numerous investigations have demonstrated the efficacy of liquid chromatography's hydrophilic interaction with a variety of polar compounds [5]. HILIC's approach to a number of previously scary separation difficulties is attracting considerable interest at the moment. The successful deployment of HILIC-technology enabled the investigation of medicines [6-14], dansyl amino acids [15], inorganic anions [16], carboxylic acid [17], sugar [18], and saccharides [19].

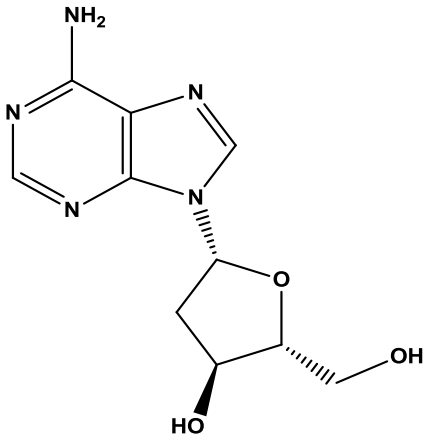

Fig.1: Structure of deoxyadenosine.

For various applications, there are several methods for nucleoside separation and assessment [20-27]. This research modeled deoxyadenosine's activities on the ZIC-HILIC exchangers ZIC1 and ZIC5.The deoxyadenosine retention was methodically tested for detecting the capacity of applicability of these columns in serum samples, and for gradient elution, optimum conditions for their separation were used. Furthermore, the effects on deoxyadenosine retention behavior of ZIC-HILIC columns chain length have not been studied.

Experimental

Conditions for chromatography

The Merck Hitachi High-Performance Liquid Chromatography System is fitted with a UV (L-4200) and a gradient pump (L-6200). The N2000 Data Workstation program was used to monitor my chromatography and analysis. The UV area with a wavelength of 254 nm was used to detect deoxyadenosine.

Two columns for the separation of 2-deoxycytidine were constructed on the Poly(styrene divinylbenzene) surface using a grafted sulfobetaine monomer (100 mm x 4 mm I.D) and a PEEK column [10, 15-17].

Chemicals

Sigma-Aldrich acquired 99% deoxyadenosine (HPLC). BDH was used to get acetic acid. Fluka was procured using sodium acetate. Sigma-Aldrich has been certified for HPLC acetonitrile. Capacity is available for ZIC1 and ZIC5 in 432 and 488 μeq g−1 [29].

RESULTS AND DISCUSSION

Separation of deoxyadenosine

The separation of deoxyadenosine was verified using two methods utilizing ZIC1 and ZIC5 columns. 5 mM sodium acetate (pH 3) and 80% ACN as mobile phase were used to obtain chromatograms, as shown in Figures 2 and 3).

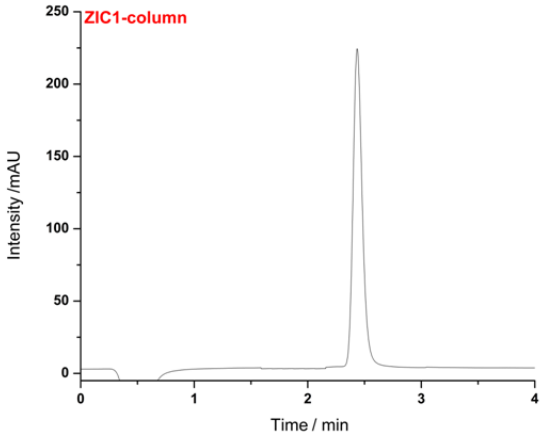

Fig.2: Separations of deoxyadenosine in ZIC1 column.

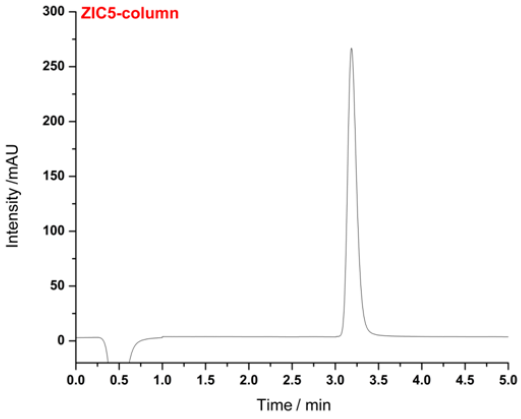

Fig.3: Separations of deoxyadenosine in ZIC5 column.

The behaviour between the deoxyadenosine and the columns shows that chain length's influence led to increasing retention factor of deoxyadenosine as illustrated in Figures 2 and 3. The reason is the methyl group rising in the two columns. The proportion of ACN varies between 60% and 95% in the eluent. The variation in acetate buffer concentrations between 5 and 25 mM at pH values of 3 to 5.5 assures that each column has distinct separative characteristics, and therefore the separation mechanism is determined.

Impact of ACN content

Increased retention factor of nucleosides due to the higher ACN percentage in the HILIC mode Additionally, hydrophilic (HILIC) activities indicate nucleosides with a reduced water content in the mobile phase. This functional distinction is owing to the nucleosides' hydrophilicity. In the ZIC1 and ZIC5 exchangers, deoxyadenosine exhibits HILIC activity as shown in Figures 7 and 8, because of its deoxyadenosine logPow of deoxyadenosine (- 2.1) [30, 31].

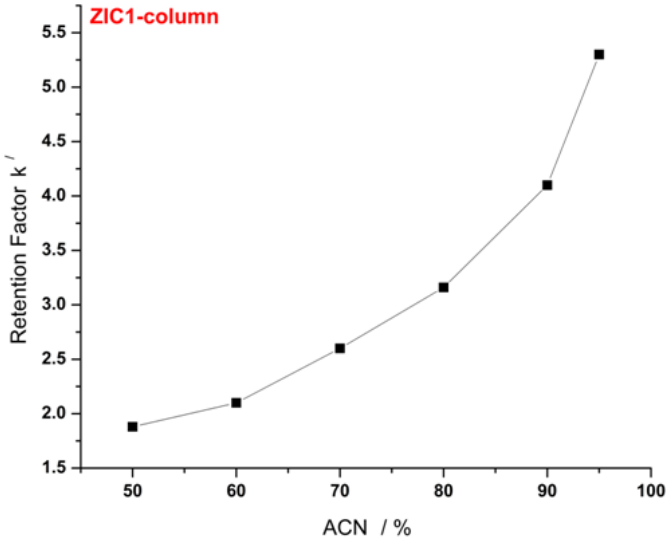

Fig.4: Impact of ACN percentage using ZIC1 column.

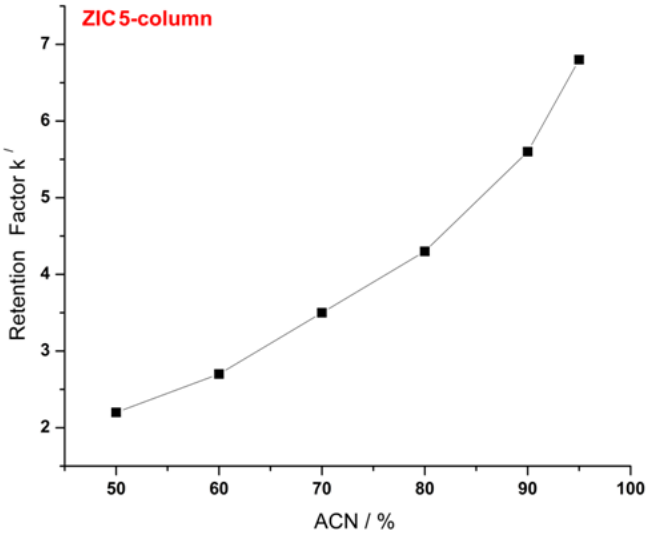

Fig.5: Impact of ACN percentage using ZIC5 column.

Eluent impact

Eluent levels were consistently elevated and intramolecular ion couples were blocked for ZIC-HILIC interactions. ACN increases the linearization of functional groupings [15]. When the buffer degree increases, the retention of nucleosides in HILIC exchangers decreases or increases [20, 32]. The exchange of cations demonstrates this [15]. As seen in Figures 6 and 7.

The deoxyadenosine retention factor value drops as the acetate buffer concentration is increased in range 5-25 mM, at conastant 80% ACN and pH 3. These slopes (0.3452 and 0.3500) are comparable to those seen in conventional ion exchange columns [33]. The value of deoxyadenosine's isoelectric point (8.68), which stays in the cationic state, is then determined by the cation exchange reaction between deoxyadenosine and ZIC-HILIC.

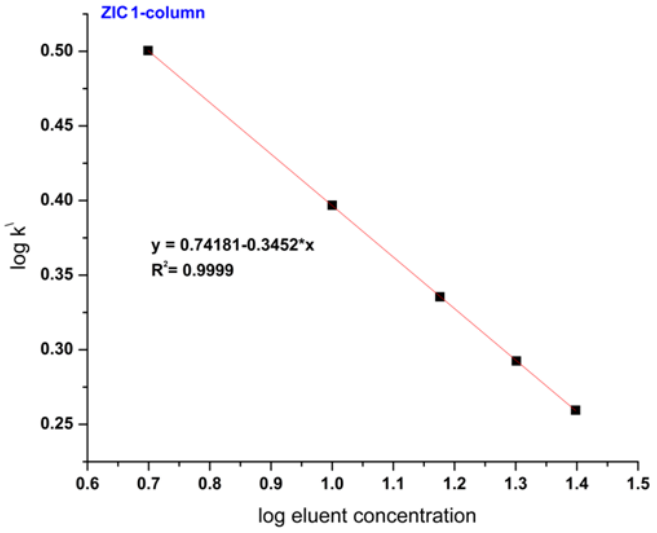

Fig.6: Eluent impact using ZIC1 column.

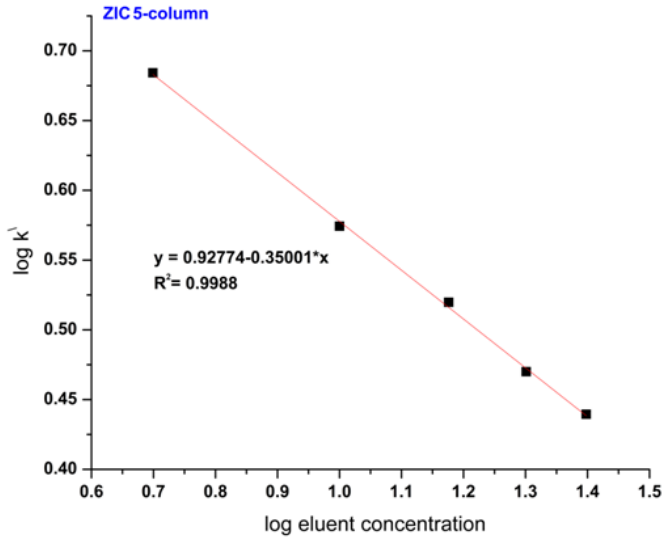

Fig.7: Eluent impact using ZIC5 column.

Eluent pH impact

Variable pH of the eluent is required to completely actualize the concept of deoxyadenosine separation. The pH of the eluent was increased in the range of 3-5.5 while maintaining ACN content at 80% with 5 mM acetate buffer.

As a result, the deoxyadenosine retention factor is decreased (Figures 8 and 9). Due to the deprotonation of the amino group in deoxyadenosine, deoxyadenosine is better preserved via the ZIC1 and ZIC5 exchangers.

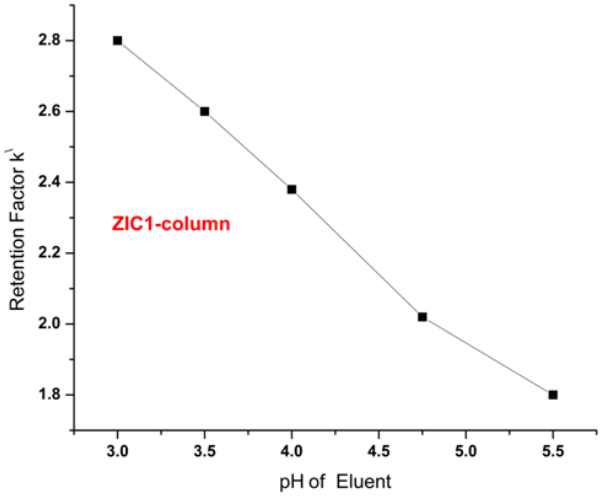

Fig.8: pH impact using ZIC1 column.

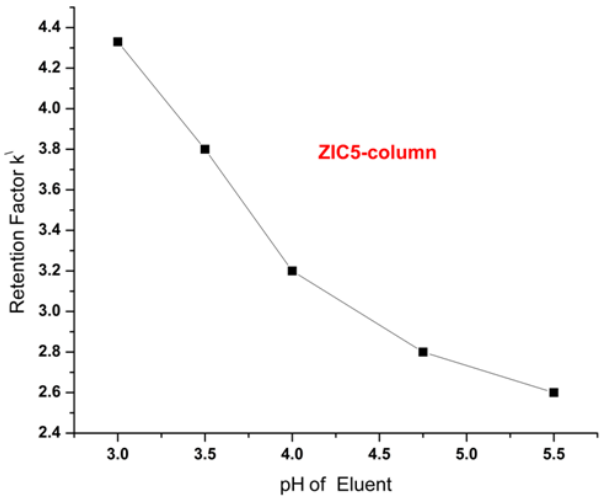

Fig.9: pH impact using ZIC5 column.

Validation

As shown in Figure 10, it is possible to observe the linearity (0.04-9 ppm) of two strategies with ZIC1 and ZIC5 exchangers with deoxyadenosine.

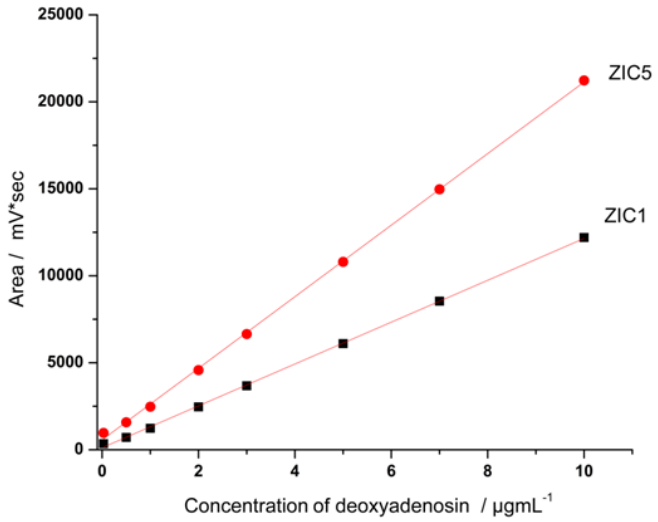

Fig.10: Standard curves for deoxyadenosine.

For extensive testing of deoxyadenosine under HILIC settings, the acceptable standard curves and statistical data in Table 1 have been used. The precision and percentages of recovery and determined on the same day. An effective technique is demonstrated by the extremely low default rates and high recuperation rates in Table 2.

Table 1: Validation result data of the deoxyadenosine.

Factor	ZIC1 column	ZIC5 column
Linearity (ppm)	0.04-9	0.04-9
Regressiona equation	y= 116.58+ 1203.11*x	y = 568.36+ 2057.32217 *x
r^2	0.9994	0.9990
LOD (ppm)	0.020	0.028
LOQ (ppm)	0.070	0.098

Table 2: Accuracy and precision of the proposed strategies.

	Intraday (n=6)				Interday (n=6)			
ZIC1 column								
deoxyadenosin Taken ppm	deoxyadenosin Found ppm	%Rec.	% $E_{rel.}$	%RSD	deoxyadenosin Found ppm	%Rec.	% $E_{rel.}$	%RSD
1.00	0.985	98.50	-1.50	1.13	0.983	98.30	- 1.70	1.28
3.00	3.02	100.66	0.66	0.93	2.988	99.60	- 0.40	0.92
5.00	4.98	99.60	- 0.40	0.77	5.02	100.40	0.40	0.82
ZIC5 column								
1.00	0.990	99.00	- 1.00	1.38	1.010	101.00	1.00	1.43
3.00	2.992	99.73	-0.27	1.27	2.983	99.43	-0.57	1.29
5.00	5.030	100.60	0.60	1.33	4.993	99.86	- 0.14	1.53

Identification of deoxyadenosine in spiking human serum samples

As demonstrated in Table 3, the proposed methodologies for quantifying in-vitro deoxyadenosine in a spiking serum with two concentrations were successful.

Table 3: Determining deoxyadenosine levels in human serum.

Deoxyadenosin Taken ppm	Deoxyadenosin Found ppm	%Rec.	$\%E_{rel.}$	%RSD n=6
ZIC1 column				
1.00	0.973	97.30	- 2.70	1.52
3.00	2.910	97.00	- 3.00	1.33
5.00	4.890	97.80	-1.20	1.51
ZIC5 column				
1.00	0.981	98.10	- 1.50	1.67
3.00	2.930	97.66	-2.34	1.32
5.00	4.830	96.60	-3.40	1.28

The comparative method's findings [34] were compared to the results of an evaluation of the ZIC1 and ZIC5 methodologies' competence and efficiency. For statistical analysis, the test findings as shown in Table 4 in variance ratios (95%) were selected.

Because the computed t and F values are not more than the theoretical value, there is no significant difference in deoxyadenosine accuracy between the two methods in a human blood sample.

Table 4: Comparison of the official method with the proposed methods.

Nucleoside	ZIC1	ZIC5	Official method	t-Test (theor.)	F-Test (theor.)
Deoxyadenosin	97.30	98.10	97.05	0.9690* (2.7764)	0.3021* (19.00)
	97.00	97.66	96.88	0.9898** (2.7764)	1.0996** (19.00)
	97.80	96.60	98.23		

* For ZIC1 method

** For ZIC5 method

CONCLUSION

The goal of this article is to explore the use of HILIC techniques to quantify the deoxyadenosine content in human serum samples. This versatility is advantageous due to the fact that HILIC trading with one to five methylene groups has at least two distinct charge-holding modes under a variety of situations. This could be a result of the ZIC5 column's geometric alignment.. According to the findings, the retention mechanism is a combination of hydrophilic and cation properties. The approaches established have been successfully applied to human serum samples.

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