RESEARCH ARTICLE

Investigate Retention Behavior of 2-deoxycytidine in Hydrophilic Interaction Liquid Chromatography

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ABSTRACT

A hydrophilic interaction chromatography has been investigated to separate 2-deoxycytidine chosen for nucleoside. A small molecule with specific features for human serum samples was 2-deoxycytidine tested. 2-deoxycytidine has been applied to self-made stationary hydrophilic phases (ZIC1 and ZIC5). The deoxycytidine (dCD) retention was investigated with varying concentrations of sodium acetate buffer, acetonitrile%, and pH. The results confirmed the hydrophilicity of 2-deoxycytidine. The exchanger retention mechanism was studied taking into account 2-deoxycytidine used for describing the interaction of hydrophilic and cation. For both ZIC1 and ZIC5 exchangers, we described and discussed the influence of chromatographic conditions (concentration of sodium acetate buffer, a percent of CAN, and pH). The two methods developed are a useful alternative to current 2-deoxycytidine separation methods.

Keywords: 2-deoxycytidine, Cation exchange interaction, Hydrophilic interaction chromatography (HILIC), Nucleoside.

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INTRODUCTION

In all living organisms, nucleosides belong to the most important nitrogen compounds. Although purine and pyrimidine nucleosides are important for nuclear acids, both metabolites involved in bioenergy processes and synthesis, including polysaccharides, phospholipids, and glycolipids, of macromolecules.[1] Nucleoside can also be absorbed and used in an exogenous way into the organism by means of food.[2] In many biological processes, nucleotides are important. Natural ingredients in all mammals, bacteria, and plant cells are the precursors of the nucleic acids that make up, and thus, are essential to the storage, transmission, and expression of genetic information in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).[3] Alpert coined the term HILIC (hydrophilic interaction chromatography) in 1990,[4] and many studies have shown the efficient technique of hydrophilic interaction between liquid chromatography and various polar compounds.[4] HILIC's approach to several previously intimidating separation problems is currently strongly attracted. The analysis of pharmaceuticals,[5-8] dansyl amino acids,[9] inorganic anions,[10] carboxylic acid,[11] sugar,[12] and saccharides,[13] by the successful implementation of HILIC technology has been achieved. dCD (Figure 1) is a component of deoxyribonucleic acid, and deoxyribonucleoside. It is similar to cytidine ribonucleoside but is removed from 2' position by a single hydroxyl group.

There are many methods for separating and estimating nucleosides in different samples.[14-19] This study was designed to describe the behavior in the homemade zwitterionic ion chromatography (ZIC)-HILIC exchangers of 2-deoxycytidine, *viz.*, ZIC1 and ZIC5. The retention of 2-deoxycytidine has been methodically examined in order to detect the applicability potential of these columns in serum samples, and the optimal conditions for their separation have been used in gradient elution. In addition, the effect of the ZIC-HILIC columns chain length on dCD retention behavior has not yet been studied.

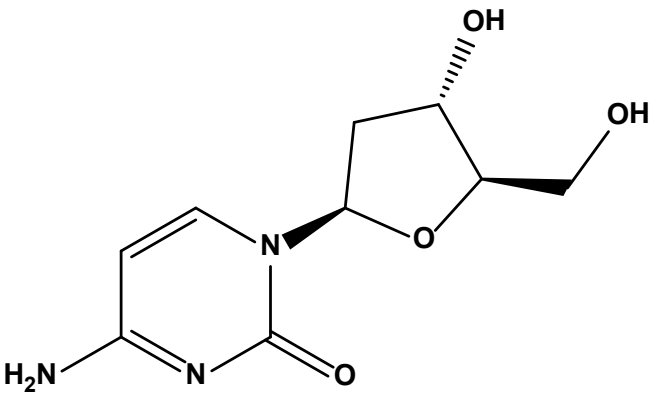

Figure 1: Structure of 2-deoxycytidine (dCD)

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MATERIALS AND METHODS

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 the chromatography and analysis. For the detection of 2-deoxycytidine, the ultraviolet region was used at a wavelength of 254 nm. ZIC1 and ZIC5 were developed on the polystyrene-divinylbenzene (PS/DVB), by means of a grafted sulfobetaine monomer (100 × 4 mm ID) and PEEK column, for the 2-deoxycytidine separation.[5,9-11] The systematic cycle of grafting reaction has been established by Raskop *et al*.[20]

Chemicals

2-deoxycytidine (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},[6] respectively.

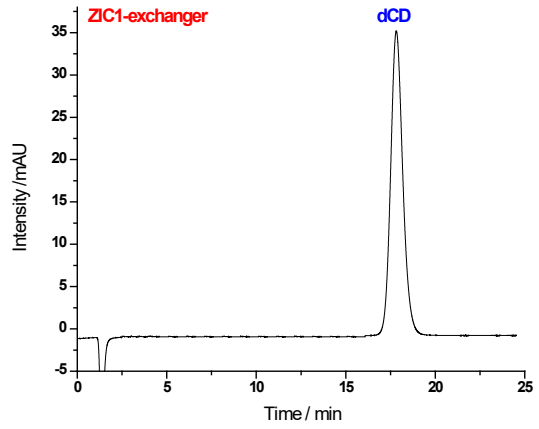

Figure 2: Chromatogram for the separations of 2-deoxycytidine (dCD) in ZIC1 exchanger

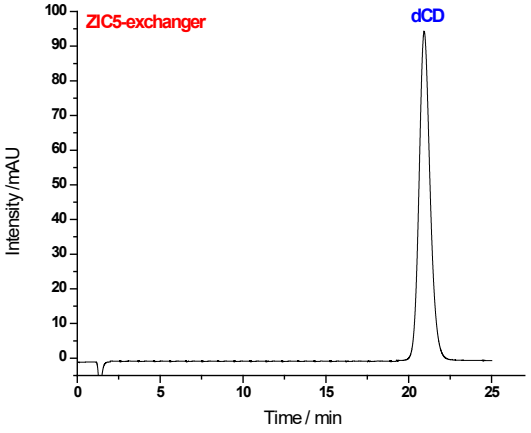

Figure 3: Chromatogram for the separations of 2-deoxycytidine (dCD) in ZIC5 exchanger

RESULTS AND DISCUSSION

Optimum Separation of dCD

A mobile phase procedure in the ZIC1 and ZIC5 exchangers tested the separation of the HILIC-mode dCD. The chromatogram is shown in Figures 2 and 3. The chromatogram was obtained in sodium acetate 5 mm (pH 4.7) and 85% of ACN.

In Figures 2 and 3, the interaction between the dCD and column is evident, as is shown by the ZIC5 exchanger; by increasing the chain length the retention time of dCD increased. It is due to the growing methyl group between charges in the ZIC column. The systemic ACN variation ranges from 50 to 95% in mobile phase compounds. The eluent concentration from 5 to 25 mm, at pH levels between 3 and 4.7, ensures that the separative properties of each interchange are assessed and thus, the separation mechanism.

Effect of dCD Retention on ACN Content

With increasing ACN content in ZIC-HILIC mode, the increase of nucleoside separations has increased. In addition, the hydrophilic (HILIC) activities indicate nucleosides that have lower eluent water content. The hydrophilicity of the nucleosides is the reason for this functional difference. In the ZIC1 and ZIC5 exchangers, dCD shows the HILIC behavior (Figures 4 and 5). The consequence is the dCD Pow log (-1.89).[21,22]

Eluent Impact on Retention of dCD

For ZIC-HILIC retention, higher levels of eluent were usually increased, which led to the deactivation of intramolecular ion pairs. So, the linearization of functional phase groups is improved when ACN is present.[9] With an increased buffer level, the retention of nucleosides has decreased or increased in HILIC exchangers.[14,23] This is demonstrated by the exchange of cations and anions.[10] The dCD retention factor decreases, as shown in Figures 6 and 7, with the NaOAc buffer rising from 5 to 25 mm and a pH of 4.7, or upto ACN 85%. This slope tends to be measured in Figures 6 and 7 on standard columns of ions exchange,[24] approximately 0.2895 and 0.3458. dCD takes a different picture as the buffer is increasing because of

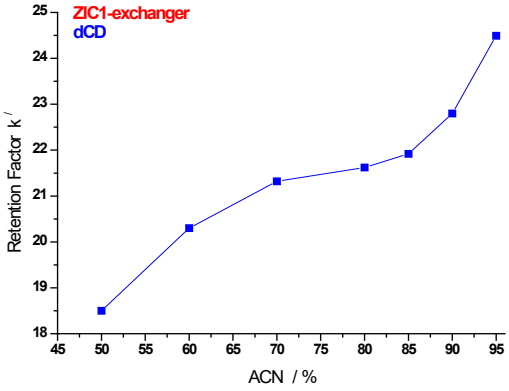

Figure 4: ACN content impact on dCD retention in ZIC1 exchanger

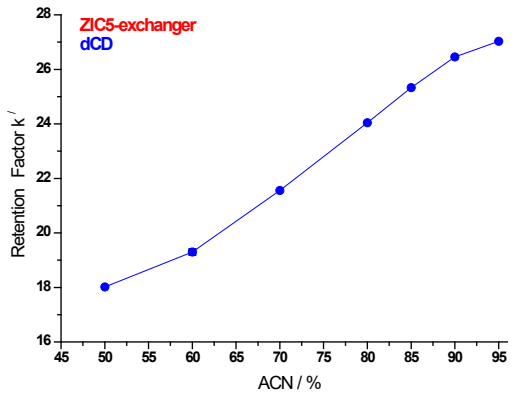

Figure 5: ACN content impact on dCD retention in ZIC5 exchanger

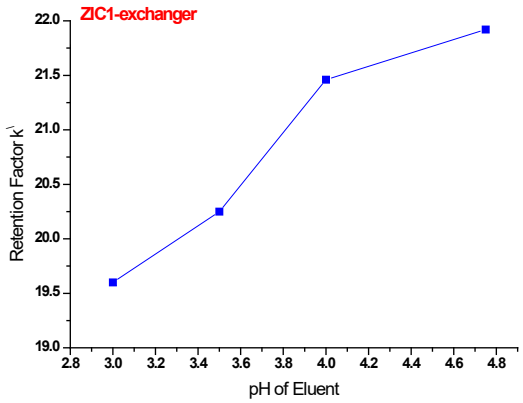

Figure 8: Eluent pH impact dCD retention in ZIC1 exchanger

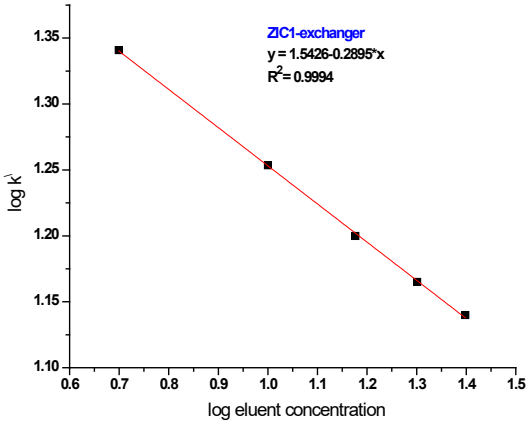

Figure 6: Eluent concentration impact on dCD retention in ZIC1 exchanger

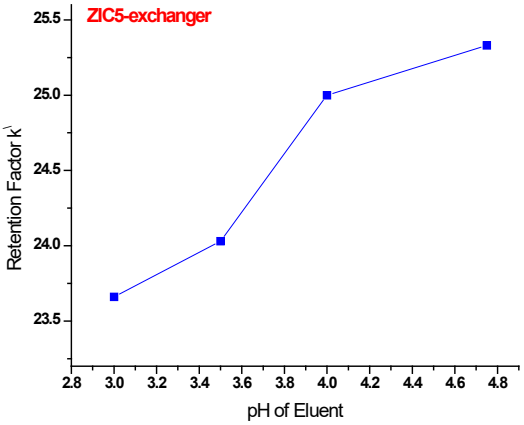

Figure 9: Eluent pH impact dCD retention in ZIC5 exchanger

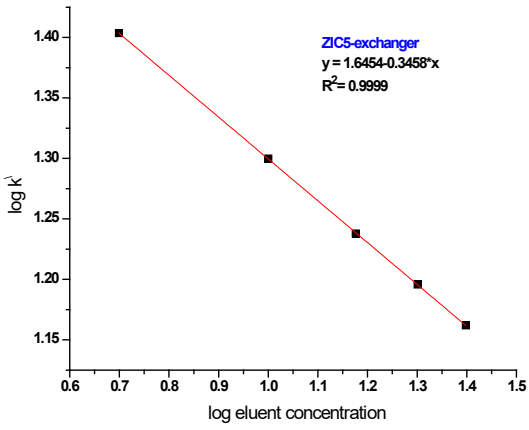

Figure 7: Eluent concentration impact on dCD retention in ZIC5 exchanger

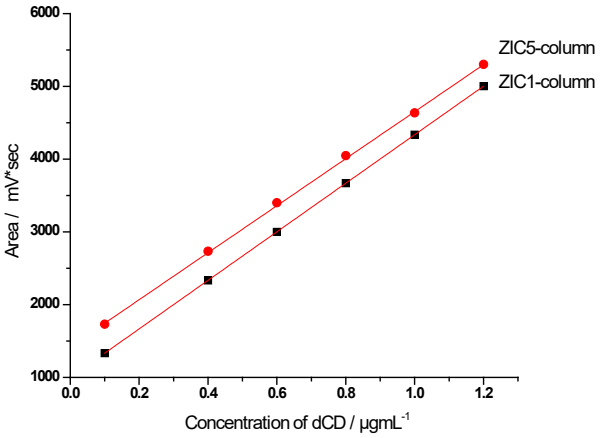

Figure 10: Calibration graph for dCD using ZIC1 and ZIC5 exchangers

two factors, when retention decreases. The core components of the exchanger and dCD. The value of the isoelectric point (9.9) in dCD, therefore, shall remain cationic. The interaction between dCD and ZIC-HILIC column was then dependent on the cation exchange.

Eluent pH Effect on dCD Retention

In order to complete the dCD separation concept, the eluent pH must be variable. dCD retention was increased from 3 to 4.75 with sodium acetate levels maintained at 5 mm and 85% of ACN, as shown in Figures 8 and 9. The preservation of

Table 1: LOD, LOQ, and linear regression data of dCD

Parameter	*ZIC1 method*	*ZIC5 method*
Linearity[a] (μg.mL^{-1})	0.1–1.2	0.1–1.2
Regression[a] equation[a]	y = 999.46 + 3,336.20 × x	y = 1,421.31 + 3,231.05 × X
r^2	0.9998	0.9983
LOD (μg.mL^{-1})	0.009	0.007
LOQ (μg.mL^{-1})	0.0315	0.0245

Table 2: Intra- and inter-day recovery of proposed approaches

	Intra-day analysis n = 5				*Inter-day analysis n = 5*			
				ZIC1 method				
dCD taken (μg.mL^{-1})	dCD found (μg.mL^{-1})	% rec.	% $E_{rel.}$	% RSD	dCD found (μg.mL^{-1})	% rec.	% $E_{rel.}$	% RSD
0.3	0.295	98.33	-1.67	1.54	0.294	98	-2	1.61
0.6	0.593	98.83	-1.17	1.32	0.597	99.5	-0.5	1.46
0.8	0.803	100.37	0.37	0.98	0.805	100.62	0.62	1.16
				ZIC5 method				
0.3	0.302	100.66	0.66	1.38	0.301	100.33	0.33	1.43
0.6	0.596	99.33	-0.67	1.27	0.598	99.66	-0.34	1.29
0.8	0.789	98.62	-1.38	1.33	0.791	98.87	-1.13	1.53

Table 3: Two approaches suggested for measurement of dCD in human serum

dCD taken (μg.mL^{-1})	*dCD found (μg.mL^{-1})*	*% rec.*	*% $E_{rel.}$*	*% RSD n = 5*
		ZIC1 method		
0.2	0.195	97.5	-2.5	1.72
0.5	0.49	98	-2	1.62
0.7	0.789	98.62	-1.38	1.3
		ZIC5 method		
0.2	0.197	98.5	-1.5	1.56
0.5	0.493	98.6	-1.4	1.43
0.7	0.792	99	-1	1.22

Table 4: Comparison of proposed ZIC1 and ZIC5 methods with dCD test method[25] for examination of t- and F-statistical tests

Name of nucleoside	*ZIC1 method*	*ZIC5 method*	*Comparison[25] method*	*t-test (theor.)*	*F-test (theor.)*
2-deoxycytidine	97.5	98.5	98.5	0.5037* (2.7764)	0.331* (19)
	98	98.6	97.55	0.9898** (2.7764)	0.0736** (19)
	98.62	99	99.5		

*For ZIC1 method; **For ZIC5 method

dCD is increased during ZIC1 and ZIC5 exchangers due to the amino group protonation in dCD.

Validation

The linearity of two methods using ZIC1 and ZIC5 exchangers for dCD; it is possible to observe (0.1–1.2 μg mL^{-1}) as Figure 10 illustrates.

For extensively checking dCD under the HILIC conditions, the corresponding calibration graphs were used and statistical results are shown in Table 1. On the same day, accuracy was measured and daily recovery percentages and RSD were determined. The relatively small defaults and high recuperation values indicate a successful approach (Table 2).

dCD Detection in Human Serum Spiked Samples

The proposed methods were successfully used with two methods in order to measure *in vitro* dCD in a spiked human serum with two concentrations; Table 3 reports the findings.

Such findings have been contrasted with results obtained by the comparative method,[25] for evaluation of the competence and efficiency of the ZIC1/ZIC5 methods. Statistic analyses were carried out on the basis of both t test results (Table 4) and

F test variance ratios (95%). The t and F values calculated do not exceed the theoretic value, which means that the exactness of the dCD determination in the human serum sample in both methods does not differ significantly.

CONCLUSION

This paper addresses the development of HILIC techniques in human serum samples for evaluating 2-deoxycytidine. A versatile separation with the advantage of having at least two different holding modes under different conditions for HILIC exchangers with one and five methylene groups among charged groups. This paper illustrates how ZIC1 and ZIC5 are treated by 2-deoxycytidine. ZIC5 with 2-deoxycytidine has been found to be preserved longer. The geometric orientation of the ZIC5 exchanger can be attributed to this. The evidence shows that the retention mechanism is both hydrophilic and cation exchange interactions. In human serum samples, the methods developed were successfully used.

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