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ORIGINAL PAPER

Retention of the N-nitrosodiethanolamine by hydrophilic interaction liquid chromatography on different polar columns: mechanism study and optimization

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Abstract
To investigate the retention mechanism of N-nitrosodiethanolamine (NDELA) in liquid chromatography, the mobile phase composition was changed on five stationary phases including silica, diol, cyano, amino, and zwitterionic. Afterward, temperature, pH, and buffer concentration effects are studied. Results demonstrate a dual hydrophilic interaction liquid chromatography (HILIC)-reversed phase (RP) mechanism for the retention of NDELA. The transition between the HILIC and RP behavior on a different column, "U-turn" points, was observed between 43 and 68% of water in acetonitrile. The depiction of the natural logarithm of retention factor vs. inverse of the absolute temperature was linear for cyano, amino, and zwitterionic columns. In contrast, it was curvature for silica and diol columns. In each column, the positive slopes indicate a negative retention enthalpy, signifying an exothermic process of transferring analytes from the mobile phase to the stationary phases being examined. In the case of buffer pH and concentration, an ammonium acetate solution with a pH of 5.7 and a concentration of 10 mM was selected for further investigation. Finally, the selectivity of this method in optimal conditions for the analysis of NDELA in shampoo has been investigated. According to the well-obtained selectivity of NDELA in shampoo and the advantages of the HILIC method, this method seems to be a suitable alternative to RP methods.

Keywords Hydrophilic interaction liquid chromatography · Dual HILIC–RP · Retention mechanism · N-nitrosodiethanolamine · Shampoo

Introduction

Even though reverse phase liquid chromatography (RP-LC) is known as a useful method for separating compounds, its efficiency in retaining highly polar compounds, particularly basic ones, can be limited unless assisted by techniques such as derivatization with a hydrophobic ligand or ion pairing, and addition of mobile phase modifiers such as surfactants in micellar conditions, ionic liquids, amines, etc. [1–3]. In addition, analysing basic compounds in silica-based RP-LC can be challenging due to the silica stationary phase instability at extreme pH values [4, 5], as well as unwanted interaction with the silanol groups of the stationary phase in high concentrations of the aqueous buffer leading to distorted peak shapes. In the case of another traditional mode in LC, normal phase (NP), not only polar and basic compounds have insufficient solubility in non-polar or less polar mobile phases, but also, the mobile phase composition is incompatible with mass spectrometric (MS) detectors.

Hydrophilic interaction liquid chromatography (HILIC), introduced by Alpert [6], is a widely recognized chromatographic technique that is highly effective at retaining highly polar compounds, especially basic analytes. HILIC achieves this without the need for ion pair reagents, using a high concentration of aqueous buffer in the mobile phase [7]. HILIC is also highly compatible with LC-MS and 2D-HPLC. One of the significant advantages of HILIC is its compatibility with various sample preparation methods, such as SPE and QuEChERS extracts, due to the organic nature of the mobile phase [8]. Additionally, the low viscosity of the mobile phase results in small back pressures, better separation efficiency, lower height equivalent to a theoretical plate

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(HETP), and more sensitivity in MS detection. The retention of basic compounds in HILIC mode requires a lower pH, which also helps to resolve the issue of peak shape, another significant challenge faced in analysing these compounds. As a result, HILIC is a well-established alternative method for separating high polar, ionic, and basic compounds [9].

The retention modelling techniques are utilized as a valuable tool in retention mechanism studies, for different practical purposes, including stationary-phase characterization and comparison, method development and optimization, and method transfer [10]. While partitioning and adsorption are the primary mechanisms for retaining polar analytes in HILIC, various equations propose different mechanisms for HILIC. The retention process in HILIC seems far more complicated than simple adsorption or partitioning [2, 11]. The characteristics of the stationary phase, the composition of the mobile phase, and the structure of the compound due to the interactions between the stationary phase, compound, and solvent, which may include electrostatic interactions, hydrogen bonding, dipole–dipole interactions, and hydrophobic interactions, greatly affect the polar compounds' retention in HILIC [12].

N-nitrosodiethanolamine (NDELA) is known as a carcinogen in category 1B [13], which because of its permeability characteristics, can be absorbed through the human skin [14], and accumulated in organs such as the bladder, liver, and kidneys, and, if induced, has chronic toxic effects [15]. NDELA is one of the most common contaminants in consumer products such as cosmetics and personal care products. Analysing NDELA in cosmetics and personal care products is a challenging task due to its high polarity and basicity. The complexity of the product matrices and the trace amounts of NDELA further add to the difficulties. However, these challenges can be overcome by using more efficient cleaning and extraction methods, accompanying other modes of liquid chromatography, and employing more sensitive detectors. There are two international standard methods, namely ISO 10130:2009 and ISO 15819:2014, that utilize RP-LC [16, 17]. Meanwhile, traditional solid phase extraction (SPE), liquid–liquid extraction (LLE), or a combination of both followed by RP-LC are the most common analytical methods for the extraction and quantitation of NDELA in cosmetic products, utilizing sensitive detection methods such as fluorescence [18], chemiluminescence [19], and mass spectrometry (MS) [19–22].

In our previous research, we aimed to minimize matrix interference by developing a suitable sample preparation method. To achieve this, the QuEChERS method was proposed, which optimized effective parameters to improve recovery percentage and matrix effect when coupled with a conventional reverse phase HPLC method featuring a water-rich mobile phase [23]. To our knowledge, no investigation has been conducted on the retention mechanism of NDELA in HILIC. Therefore, in this research, this investigation was carried out on five different columns along with data fitting to various proposed equations for more popular mechanisms. Also, the effect of temperature, buffer concentration, and pH on the retention of NDELA was examined in all columns. Finally, the selectivity of this method under optimal conditions for analysing NDELA in shampoo was evaluated, using blank, fortified blank, and commercial shampoo samples.

Experimental

Chemicals

NDELA was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Ammonium acetate and HPLC grade acetonitrile (ACN) were obtained from Biochem Chemopharma (Cosne-Cours-sur-Loire, France). The dispersive SPE sorbents used included primary secondary amine (PSA; 50 µm) from Agilent (Bellefonte, PA, USA), silica (15 µm) from Merck (Darmstadt, Germany), and C18 (10 µm) from YMC (YMC*GEL Kyoto, Japan). Anhydrous magnesium sulfate ($MgSO_4$ > 98%) and sodium chloride (NaCl > 99.5%) were purchased from Chem-Lab NV (Zedelgem, Belgium) and Arman Sina (Tehran, Iran) respectively. Ultra-pure deionized water (18.2 MΩ cm), was acquired using a Direct-Q system (Millipore, St Quentin, France). During the evaluation of the HILIC method suitability, a blank shampoo sample containing no precursors of NDELA, formulated by the R&D department of Goltash (Isfahan, Iran) was utilized.

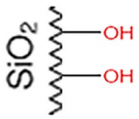
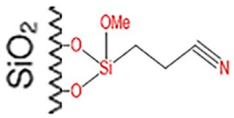
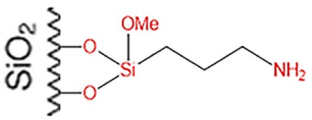
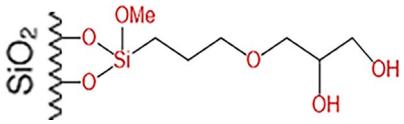
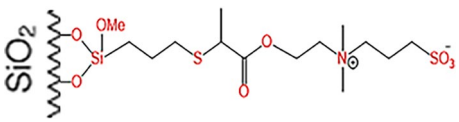
Instruments

During the sample preparation process, various apparatus were used including an ultrasonic bath (Wise Clean, Germany), a vortex mixer (WiseMix VM-10, Witeg, Germany), and a centrifuge (Behdad Universal Centrifuge, Iran). All experiments were carried out using a YoungLin 9100 series liquid chromatograph (Young-Lin, Anyang-si, Korea), equipped with a vacuum degasser (YL9101), a quaternary pump (YL9110), a thermostat column compartment (YL9131), a UV detector (YL9120) and a manual injector with a 20 µL loop (Rheodyne, Cotati, CA, USA). The YL-Clarity software (version 3.0.4.444) was used for instrument control, data acquisition, and analysis.

Standard solution and sample preparation

A stock solution of NDELA was prepared in ACN using a standard to achieve a concentration of 5000 µg mL^{-1}. Working standards were then prepared by diluting the stock solution with the mobile phase. Blank and expired shampoo

Table 1 Some of the important features of investigated columns

Column/manufacturer	Phase type (Abbreviation)	Dimensions (mm)	Particle size (µm)	Pore size (A°)	Surface area (m^2 g^{-1})	Structure
Zorbax RX-SiL/Agilent	Silica (Si)	4.6×250	5	100	330	
Eurospher/Knauer	Cyano (CN)	4.6×250	7	100	350	
Spherisorb®/Waters	Amino (NH_2)	4.6×250	5	100	350	
ProntoSiL/Bischoff	Diol (Diol)	4.6×150	3	120	300	
Nucleodur® HILIC/ Macherey–Nagel	Sulfobetaine (ZIC)	4.6×150	5	100	350	

samples were prepared according to a previously optimized method in our laboratory [23]. The procedure involved transferring 2 g of rigorously weighed samples to a volumetric flask and filling it with distilled water up to 5 mL. The mixture was shaken vigorously for 3 min using a vortex mixer at maximum speed, and then 3 mL of the mixture was transferred to a falcon. An adequate 6 mL of ACN was added as the extraction solvent, followed by vortexing for 3 min and sonicating for 10 min. The LLE was completed by adding the mixture of salts, including 1.8 g $MgSO_4$ and 0.6 g NaCl, and shaking vigorously for 3 min. The sample was centrifuged at 4000 rpm for 10 min. The organic upper layer (5 mL) was transferred to another falcon. Various amounts of dSPE sorbents, including 300 mg $MgSO_4$, 100 mg PSA, 100 mg C18, and 100 mg silica, were added to the falcon. The clean-up step was completed by vortexing for 3 min, followed by centrifugation at 4000 rpm for 10 min. The supernatant was collected for further analysis by HPLC. The fortified sample was prepared by spiking the blank shampoo sample to achieve a final concentration of 500 ng mL^{-1}. All stock and working solutions were stored at 4 °C and protected from light.

HPLC method

Five different particle-packed columns, including silica (Si), diol (Diol), cyano (CN), amino (NH_2), and zwitterionic (ZIC) columns, were used to carry out chromatography assessments. To investigate and compare the retention mechanisms of NDELA on these columns, the composition of the mobile phase (water/ACN) was varied from 0 to 100% water. The temperature effect on retention factor of NDELA (k) and efficiency parameters of columns, such as theoretical plates per meter (t.p./m), peak width at half height (w0.5), and asymmetry, were examined by changing the column temperature from 25 to 45 °C while maintaining the composition of mobile phase constant (water/ACN at 10:90 v/v). The retention data for each column was recorded, and van't Hoff plots were constructed. Additionally, the impact of pH and concentration of buffer on the retention times in the mobile phase of ammonium acetate/ACN (10:90, v/v) was investigated. The pH and concentration of ammonium acetate were varied from 3 to 6 and 5 to 20 mM, respectively. Throughout the experiments, the flow rate was kept constant at 0.8 mL min^{-1}, UV detection was done at 234 nm, and an injection volume of 20 µL was used. The average retention factors were obtained based on three injections for each experiment.

Data analysis

In order to determine the most appropriate equation to describe the empirical data, non-linear least square models were constructed using the Solver Add-in Excel (Microsoft Excel 2016 MSO (16.0.4266.1001) 64-bit). The models

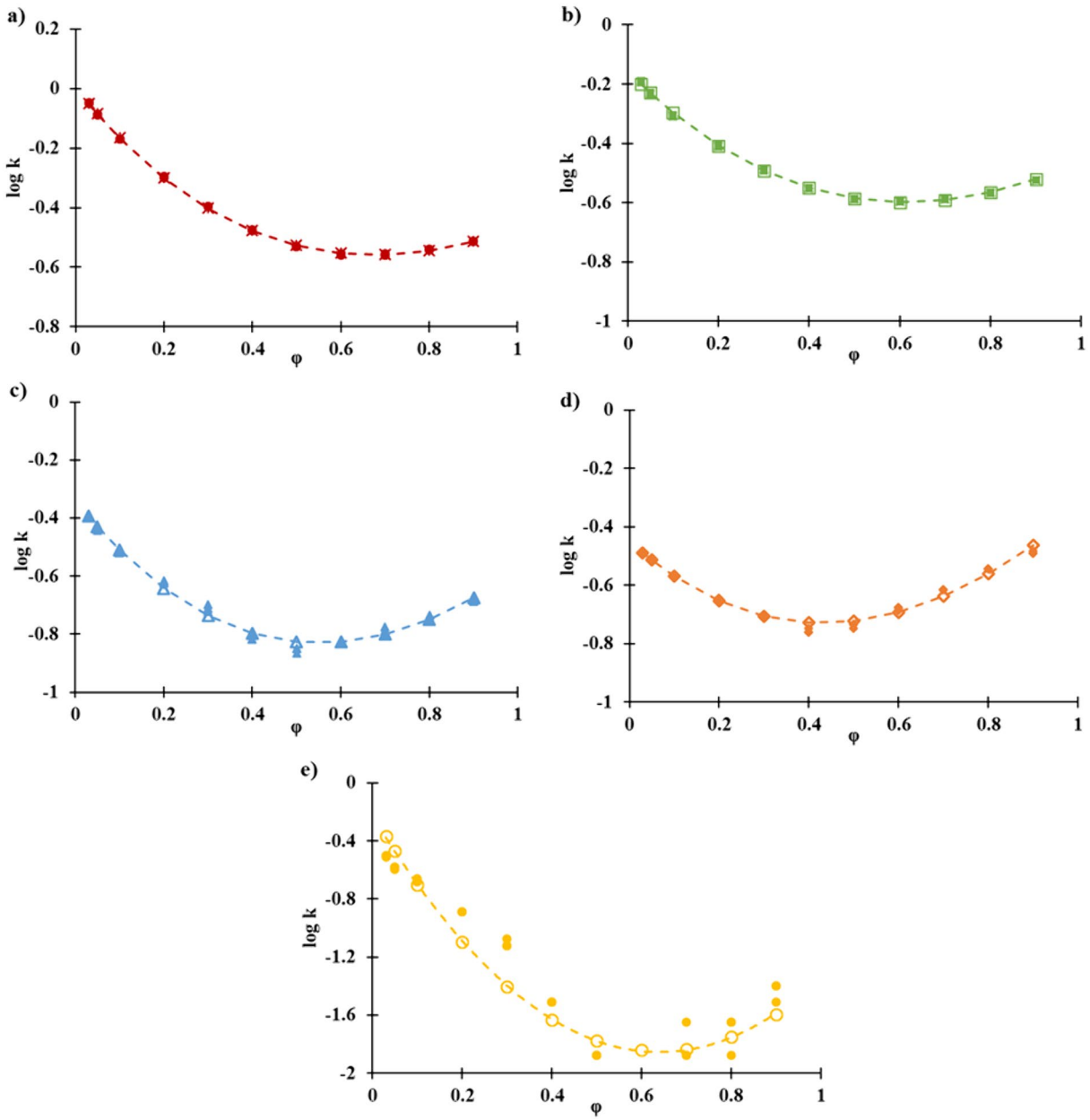


Fig. 1 Effect of the volume fraction of water in the mobile phase on the retention factor of NDELA obtained using **a** ZIC, **b** NH_2, **c** Si, **d** CN, and **e** Diol columns. Filled and empty symbols represent experimental and calculated values based on Eq. (5) respectively

were built based on the generalized reduced gradient (GRG) method, which minimized the sum of squared residuals (SSR) as a criterion for evaluating the goodness of fit. Additionally, the standard errors (SEs) were multiplied by a suitable constant obtained from statistical tables to investigate the confidence intervals of the fitted models. The SE was calculated using Eq. (1)

$$SE = \sqrt{\sum (y_i - \hat{y}_i)^2 \Big/ n - p} \quad (1)$$

where yi and $\hat{y}i$ represent the experimental and theoretical values of y, respectively, the variable n denotes the number of observations, while p represents the number of parameters in the fitted equation.

Table 2 The obtained coefficients of the used equations were determined utilizing non–linear regression in the mobile phase contents 3–90% v/v of the water

Columns	$\log k = a_1 + m_{RP} \cdot \varphi_{H_2O} - m_{HILIC} \cdot \log \varphi_{H_2O}$				
	a_1	m_{RP}	m_{HILIC}	SE[a]	$R^{2, b}$
ZIC	−0.6413	0.0602	0.4160	0.0378	0.9631
NH_2	−0.8500	0.3000	0.5000	0.0460	0.9481
Si	−1.1232	0.3912	0.5060	0.0543	0.8874
CN	−1.1100	0.5500	0.4600	0.0595	0.6789
Diol	−1.6000	−1.0000	1.0000	0.4494	0.8210

Columns	$\log k = a_2 + m_{RP} \cdot \varphi_{H_2O} - m_{HILIC} \cdot \log(1 + b \cdot \varphi_{H_2O})$						
	a_2	m_{RP}	m_{HILIC}	b	SE	φ_{min}	R^2
ZIC	0.0046	4.2034	21.1126	0.6651	0.0039	0.68	0.9997
NH_2	−0.1556	6.2717	44.4255	0.4061	0.0055	0.61	0.9989
Si	−0.3381	7.5135	46.5071	0.4679	0.0179	0.55	0.9897
CN	−0.4492	7.0290	42.2031	0.4593	0.0173	0.43	0.9724
Diol	−0.2131	42.3736	556.0535	0.1976	0.6130	0.64	0.9343

[a]SE: Standard error, [b]The correlation coefficient between the theoretical log k and experimental log k

Results and discussion

Study of the retention mechanism

A comprehensive understanding of the retention mechanism in HILIC plays a crucial role in optimizing the conditions for analysing target analytes. The stationary phase and mobile phase composition significantly influence the retention behaviour of analytes. To investigate this, the mobile phase composition was altered on five different columns, selected from diverse categories based on the charge characteristics of the functional groups, namely, neutral (Diol and CN), negatively charged (Si), positively charged (NH_2), and zwitterionic phases (ZIC). Table 1 outlines some of the critical features of these columns. The obtained retention factors on these columns were plotted against the water volume fraction, φ. Many different mechanisms have been proposed for HILIC, including adsorption, partition, dual RP-HILIC, etc., described by various equations. Equations (2) and (3) were proposed in the past for a limited range of organic-aqueous content, and they were believed to illustrate analyte retention by the adsorption and partition mechanisms, respectively [24, 25].

$$\log k = a_1 - m_{HILIC} \cdot \log \varphi \tag{2}$$

$$\log k = a_2 - m_{HILIC} \cdot \varphi \tag{3}$$

where, a_1 (extrapolated, not real) is the logarithm of the retention factor of the analyte in pure water (at $\varphi = 1$), and a_2 (extrapolated, not real) is the logarithm of the analyte retention factor in a pure organic solvent. The parameter φ is the volume fraction of the more polar (stronger) solvent in the mobile phase, water here. m_{HILIC} is the elution strength factor of solvent, characterizing the effect of the polar solvent concentration on the rate of retention decrease [26, 27]. The data fitting results revealed that neither Eq. (2) nor Eq. (3) proved to be satisfactory across all columns (data not displayed). This same result was also noted in prior reports for different compounds. Consequently, more intricate equations that accounted for mixed-mode interactions, such as a dual HILIC/RP mechanism, were introduced [28]. In this particular case, we observed that the analyte retention initially decreased by increasing the aqueous phase content in the mobile phase, until it reached its minimum retention point. After that, the analyte was retained again as the water content was increased, leading to a mechanism transition from HILIC to RP. This mechanism results in a U-shaped profile when depicting the logarithm of the retention factor, log k, versus the volume fraction of water, φ, which is similar to what we obtained in Fig. 1. Equation (4) is capable of describing the relationship between retention factor and aqueous volume fraction in the presence of a dual HILIC/RP mechanism:

$$\log k = a_1 + m_{RP} \cdot \varphi_{H_2O} - m_{HILIC} \cdot \log \varphi_{H_2O} \tag{4}$$

The parameter "a" which is a system constant, depends on the analyte and the organic solvent type. The m_{RP} represents the influence of an increase in aqueous phase concentration within the mobile phase on retention caused by the RP mechanism in aqueous-rich mobile phases. Conversely, the m_{HILIC} value demonstrates how the presence of water can

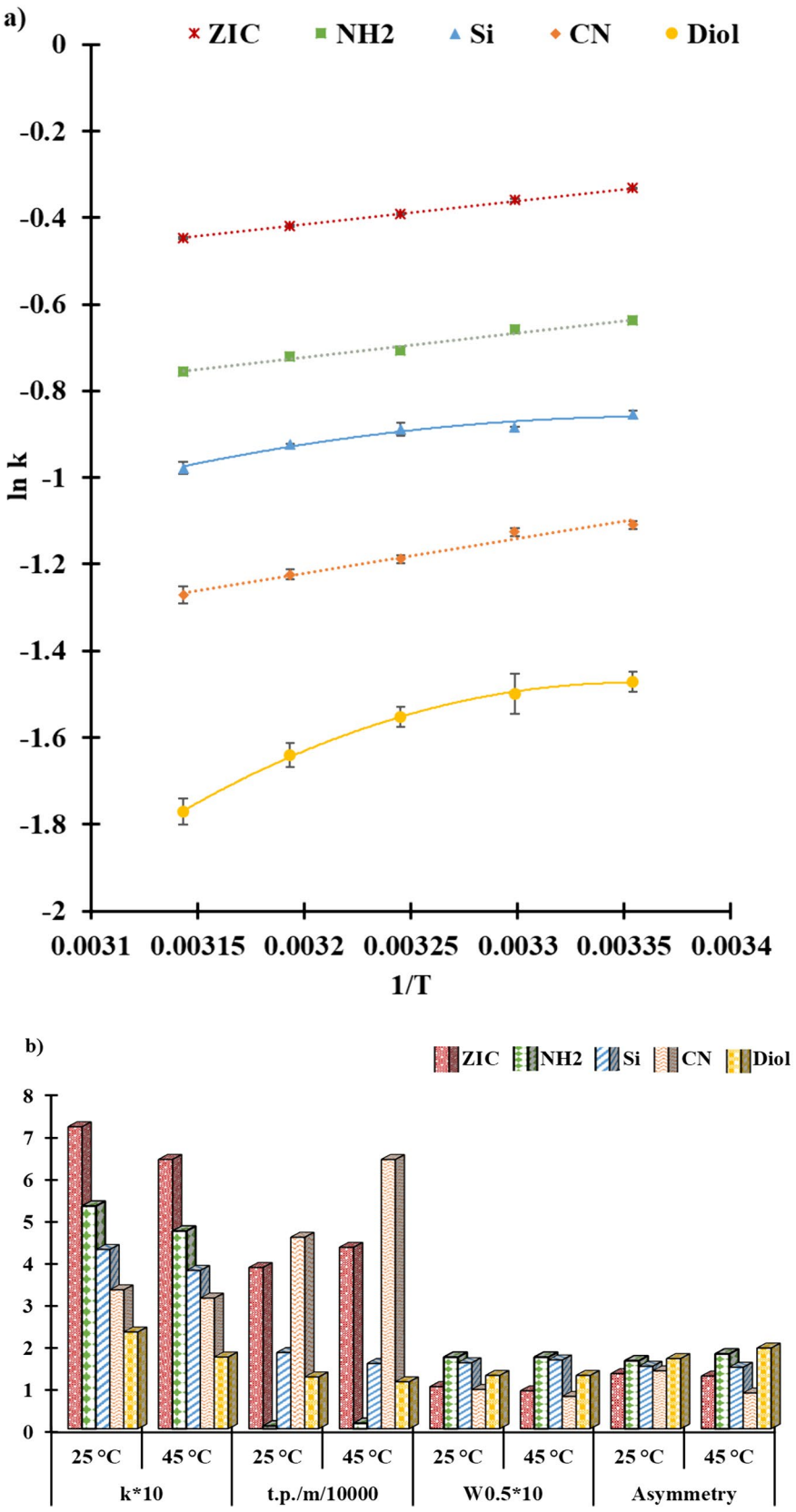


Fig. 2 **a** The van't Hoff plots for NDELA on ZIC, NH_2, Si, CN, and Diol columns, **b** The effect of temperature on NDELA's retention factor (k) and column efficiency parameters including theoretical plates per meter (t.p./m), peak width at half height (w0.5), and asymmetry. Mobile phase: water/ACN (10:90, v/v), flow rate 0.8 mL min^{-1}

Table 3 The van't Hoff equations and retention enthalpy of NDELA on five different columns, mobile phase: water/ACN (10:90, v/v), the flow rate of 0.8 mL min^{-1}

Column	Equation	R^2	ΔH (kJ mol^{-1})
ZIC	$\ln k = 547.40\ (1/T) - 2.12$	0.9996	−4.55
NH_2	$\ln k = 567.91\ (1/T) - 2.54$	0.9786	−4.72
Si	$\ln k = -2.23 \times 10^6\ (1/T)^2 + 1.50 \times 10^4\ (1/T) - 26.22$	0.9682	−17.19*
CN	$\ln k = 796.69\ (1/T) - 3.77$	0.9763	−6.62
Diol	$\ln k = -6.61 \times 10^6\ (1/T)^2 + 4.43 \times 10^4\ (1/T) - 75.82$	0.9992	9.16*

*at T = 35 °C

lead to a decrease in retention in mobile phases with high organic content. However, it's worth noting that Eq. (4) may not be effective when water concentrations are exceedingly low (< 2–5%). To rectify this, an additional empirical term, b, can be added to Eq. (5) to improve the accuracy of retention data over a wide range [28].

$$\log k = a_2 + m_{RP} \cdot \varphi_{H_2O} - m_{HILIC} \cdot \log(1 + b \cdot \varphi_{H_2O}) \quad (5)$$

Table 2 presents the coefficients that were obtained as a result of our empirical analysis. The data indicates that Eq. (4) and Eq. (5) are highly compatible with the data, and the inclusion of the "b" phrase enhances the retention mechanism description across a wide range of water concentrations. It should be noted that ZIC and NH_2 were identified as the best-fitting equations, whereas none of the equations could provide an acceptable description for the retention behaviour of NDELA on the Diol column. By plotting the theoretical log k obtained from Eq. (4) and Eq. (5), against experimental log k, the correlation between them was determined and is also presented in Table 2. Based on these results (also Fig. S1 and S2), it is noteworthy that the best R^2 values were derived from Eq. (5) belonging to ZIC and NH_2 with respective values of 0.9997 and 0.9989. The R^2 values for the other columns range from 0.9343 to 0.9897. Additionally, the residual plots constructed from the fitting of Eq. (4) indicate the inappropriateness of fitting (Fig. S3), whereas those obtained from Eq. (5) indicate that there is no systematic error except for the Diol column (Fig. S4). It is also noteworthy that in all the columns, m_{HILIC} is significantly larger than m_{RP}, which suggests that water has a greater impact on decreasing the retention in highly organic mobile phases than its effect on increasing retention in highly aqueous mobile phases.

In the dual HILIC-RP mechanism, the retention will be minimum in the "U-turn" point. This point's composition (φ_{min}) is typically influenced by the analyte's polarity and the stationary phase type and represents the transition from the HILIC to the RP mechanism. This parameter can be acquired from the derivation of Eq. (5) or obtained by Eq. (6) for different columns [29, 30].

$$\varphi \min = \left(0.434 \cdot m_{HILIC}/m_{RP}\right) - (1/b) \quad (6)$$

The transition from HILIC to RP behaviour was found to occur at 43% and 68% on the CN and ZIC columns respectively, while, for the other columns, this transition ranged from 55 to 64%. Notably, the mechanism transition occurred in the same region as that obtained from the derivatization of Eq. (6), and showed a perfect agreement with the theoretical φ_{min} as demonstrated in Fig. 1. It is worth mentioning that the ZIC column exhibited a broader range of HILIC behavior.

The effect of temperature

In HILIC mode, column temperature can affect significantly mobile phase viscosity, analyte diffusivity, and analyte transferring enthalpy between stationary and mobile phases. This can result in alterations to both the polar analytes retention and the column efficiency [31]. In addition, insight into retention interactions can be obtained from the relationship between the retention factor and temperature [31, 32] which can be described by the van't Hoff equation (Eq. 7) [33]:

$$\ln k = -\Delta H^0/RT + \Delta S^0/R \quad (7)$$

In this case, the standard enthalpy (ΔH^0) and entropy (ΔS^0) changes are temperature-independent. The value of ΔH^0 can be calculated using the slope of the line, which is then multiplied by the gas constant (R). The van't Hoff plot, as described by Eq. (8) [33], exhibits curvature behavior which indicates that there are multiple forces currently at play, and several interactions (such as electrostatic interactions, polar interactions, and partitioning) may be responsible for the retention under the experimental conditions [32].

$$\ln k = a + b/T + c/T^2 + \ldots \quad (8)$$

In such circumstances, the standard enthalpy (ΔH^0) and entropy (ΔS^0) changes are temperature-dependent and can still be determined at specific temperatures. The Eq. (9) can obtain ΔH^0 [33]:

$$\Delta H^0 = -R(b + 2c/T) \quad (9)$$

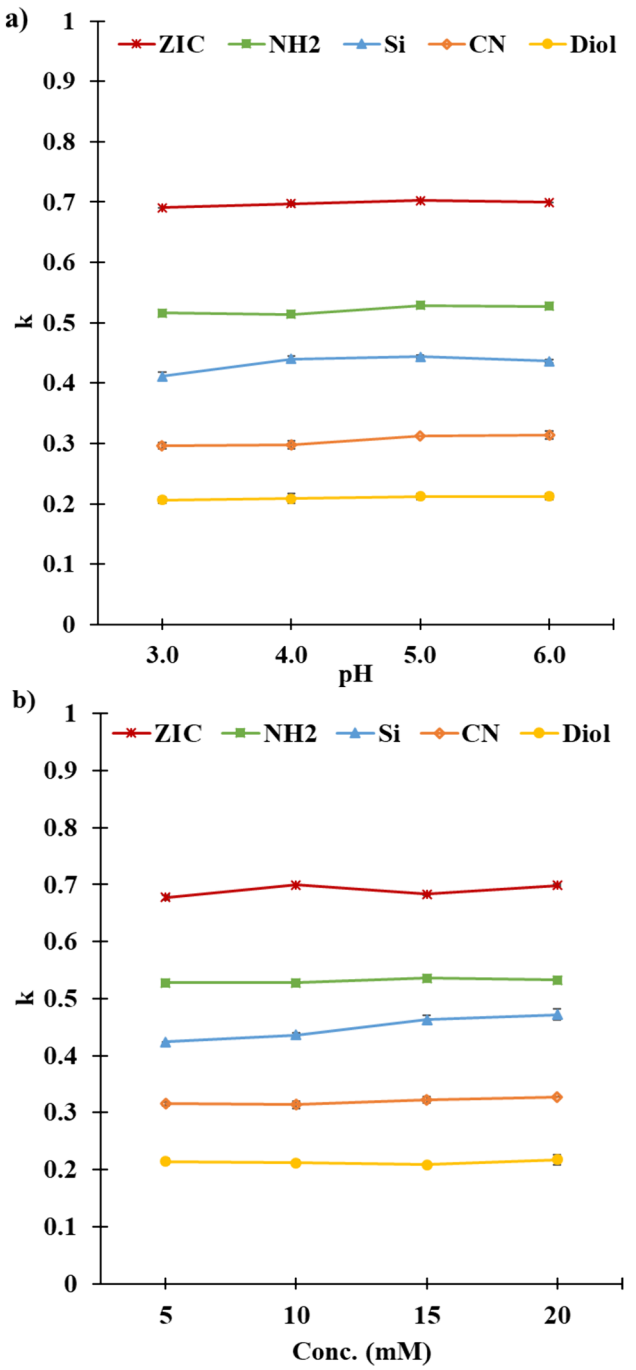


Fig. 3 **a** Effect of pH on the retention of NDELA on five different columns, mobile phase: ammonium acetate 10 mM/ACN (10:90, v/v), flow rate 0.8 mL min^{-1}, T = 35 °C. **b** Effect of salt concentration on the retention of NDELA on five different columns, mobile phase: ammonium acetate pH = 5.7/ACN (10:90, v/v), flow rate 0.8 mL min^{-1}, T = 35 °C

In the present study, the influence of temperature was considered across a temperature range of 25–45 °C in the HILIC behaviour region, and the findings are presented in Fig. 2. According to Fig. 2a, it can be observed that the retention decreases as the column temperature rises, indicating a negative retention enthalpy and an exothermic process of transferring analytes from the mobile phase to the stationary phase. The van't Hoff plots for NDELA were predominantly linear on NH_2, CN, and ZIC columns but showed curvature on Si and Diol columns (Table 3). The enthalpy values of NDELA were determined at − 4.72, − 6.62, and − 4.55 kJ mol^{-1} on NH_2, CN, and ZIC columns, respectively. Further, ΔH^0 was determined at − 17.19 and 9.16 on Si and Diol columns, respectively, at 35 °C. It is worth noting that increasing column temperature from 25 to 45 °C can enhance HPLC performance, resulting in an increase in column plate number per meter (t.p./m) from 12 to 96% for ZIC and NH_2 columns, respectively. It is worth noting that the diffusion coefficient may increase as the temperature rises, leading to narrower peaks from 10% for ZIC to 17% for the CN column. Additionally, higher temperatures may result in more symmetrical peaks. A summary of all the findings can be found in Fig. 2b.

The effect of buffer pH and concentration

The mobile phase's pH and ion strength are critical parameters that were controlled by buffers [34]. Moreover, Buffers play a crucial role in stabilizing the charge groups of ionogenic species present on both the stationary phase and analyte [35]. In this study, the effect of buffer concentration and pH on the retention of NDELA was investigated by utilizing the ammonium acetate at pH values ranging from 3 to 6 and concentrations ranging from 5 to 20 mM. It is worth mentioning that a pH value less than 3.0, can cause cleavage of bonded ligands, while a pH value greater than 6.0, can result in solubilization of silica particularly in bare silica columns that are unprotected by bonded ligands [36]. Also, a further increase in the buffer concentration of 20 mM is not possible due to the solubility limit of buffers in a high proportion of ACN in the mobile phase. Beforehand, it has been concluded that the HILIC mechanism dominated in the mobile phase of water/ACN (10:90 v/v). Multiple interactions, for instance, electrostatic interaction, hydrogen bonding, and hydrophilic partitioning, among others, may be involved in the HILIC mechanism. The retention/separation in HILIC mode is influenced by the polarity and ionization of both analytes and stationary phases. NDELA, being a strong basic compound, is protonated under all experimental pH ranges. Consequently, its retention on various columns depends on the behaviors of those columns, including their hydrophilic interaction, coulombic attraction, and repulsion with their charged groups at different pH levels.

The obtained results indicate that the use of buffers instead of water resulted in a retention factor increase of 36% to 45%, 10% to 16%, and 2% to 3% in the pH range of 3–6 on Si, CN, and ZIC columns, respectively. On the contrary, a slight decrease in retention factor was observed

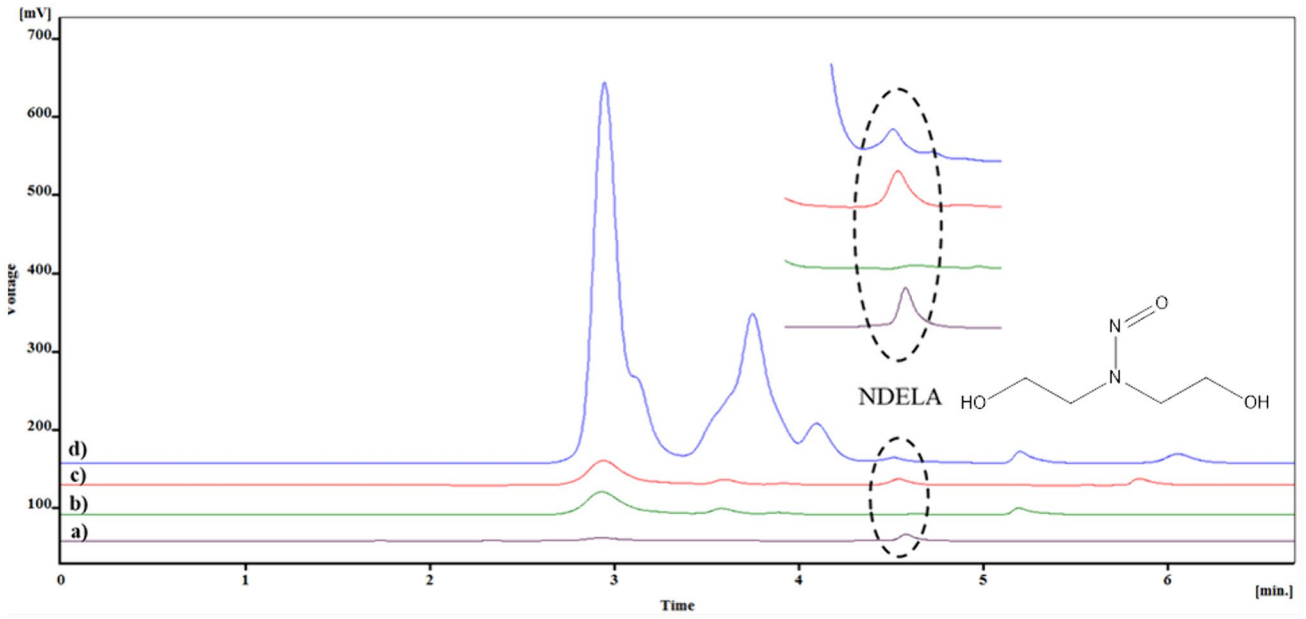


Fig. 4 Obtained chromatograms of **a** standard solution of NDELA at 500 ng mL^{-1} concentration level **b** blank, **c** fortified blank (at 500 ng mL^{-1} concentration level of NDELA), and **d** expired commercial shampoo samples under optimum conditions of ammonium acetate 10 mM pH = 5.7/ACN, 3:97 v/v with a flow rate of 0.6 mL min^{-1} at 35 °C

on NH_2 and Diol columns. In order to better understand the impact of buffer concentration and pH on different columns, the behavior of each factor was examined in the utilized concentration and pH range.

The Si column, characterized by an increased number of negatively charged silanol groups with rising pH, showed an increased retention factor (Fig. 3a.) due to enhanced ionic interactions with the positively charged NDELA [36].

Conversely, the CN column, lacking hydrogen bonding capability and hydrophilicity, was unable to retain most polar compounds sufficiently. Although the CN column was not charged, its residual silanol groups could carry negative charges at pH values above 4–5 [32], thus leading to an increase in NDELA retention upon pH elevation. As can be seen in Fig. 3a, the obtained results confirm the aforementioned interpretation.

The nucleodur ZIC column, with a 1:1 ratio of positively (quaternary ammonium) and negatively (sulfonic acid) charged groups [32], exhibited the largest retention factor, which was minimally affected by changes in pH. This suggests that the column is robust in this pH range, as illustrated in Fig. 3a. The bulk layer of water which can be formed by the water adsorption on the sulfoalkylbetaine bonded phases by hydrogen bonding becomes a part of the stationary phase and controls the retention mechanism largely. It should be pointed out that the sulfonic acid group's negative charge located at the sulfobetaine phase's distal end [37, 38], may also be responsible for the electrostatic interactions with positively charged NDELA.

In the case of the NH_2 column, both the hydrogen bonding and electrostatic repulsion might be involved in NDELA's retention mechanism. Insomuch, NDELA has a hydrogen bond acceptor count of "5" [39], it seems that hydrogen bonding could overcome electrostatic repulsion.

Finally, the Diol column, a highly polar column with hydrogen bonding capability, retained NDELA to a lesser extent, with no significant change observed in its retention upon pH variation due to the lack of ionizable groups.

Also, upon increasing the concentration of salt, the more solvated salt ions were driven out into the water-enriched layer formed on the stationary phase. Consequently, the volume of the water layer increased, leading to an increase in retention [40]. Moreover, an accompanying thickening of the water layer on the stationary phase occurs through hydration [34]. As shown in Fig. 3b, these effects were observed only for the Si column. It is noteworthy that an increase in salt concentration has a dual effect on the water layer. It increases in volume while reducing the electrostatic repulsion of positively charged column surfaces and NDELA. Despite this, the retention factor does not undergo significant changes.

As mentioned above, the HILIC has advantages in the analysis of basic and polar compounds, which seems to be able to overcome the challenges of NDELA analysis in shampoo. Consequently, the selectivity of this method in the HILIC region for analyzing NDELA in shampoo was evaluated, using the blank, fortified, and commercial shampoo samples after preparation by QuEChERS. For this purpose,

two columns, ZIC and NH_2, were selected for further investigation, as they retained NDELA more effectively. The mobile phase composition of ammonium acetate 10 mM pH = 5.7: ACN, in three levels of 5:95, 3:97, and 0:100 v/v by the flow rate of 0.8 mL min^{-1}, and 35 °C as column temperature were used to evaluate. Although complete separation of NDELA from the matrix was not observed in any mobile phase composition of 0:100 and 5:95 v/v, relatively better separation was achieved in the composition of 3:97 v/v. As can be seen in Fig. 4, by reducing the flow rate to 0.6 mL min^{-1}, complete separation of NDELA's peak from the matrix was achieved. Finally, the separation of NDELA from other components in the sample matrix is demonstrated in Fig. 4c, d. The specificity was confirmed by spiking NDELA with an appropriate level. Based on these results, HILIC can be a suitable alternative analysis method to overcome challenges in the determination of NDELA in complex matrices. However, it is important to perform validation, calculate uncertainty, and compare the obtained LOQ with the allowable limits of NDELA in shampoo before replacing current methods.

Conclusion

This study aimed to investigate the retention behavior of NDELA, a small, polar, and basic compound, in different columns. The results indicated a dual HILIC-RP mechanism for retaining NDELA, which was confirmed by solver data fitting. The transition between the HILIC and RP behavior, commonly known as "U-turn" points, demonstrated a perfect agreement with the theoretical φ_{min}. Additionally, the temperature effect was considered in the HILIC behavior region from 25 to 45 °C. The van't Hoff plots for NDELA were mostly linear on the NH_2, CN, and ZIC columns, but were curvature on the Si and Diol columns with a positive slope for all columns. Substituting water with a buffer led to an increase in the retention factor on most columns. Based on the results, an ammonium acetate solution with a pH of 5.7 and a concentration of 10 mM was selected for further investigation. Finally, the potential of HILIC methodology for retention of the NDELA was investigated for its analysis in shampoos by using the blank, fortified blank, and expired shampoo samples containing a significant amount of NDELA upper allowable limit. The obtained results showed complete separation of NDELA's peak from the shampoo matrix in ammonium acetate 10 mM pH = 5.7/ACN 3:97 v/v at the flow rate of 0.6 mL min^{-1} and 35 °C. Based on the obtained results, it can be inferred that Hydrophilic Interaction Liquid Chromatography (HILIC) presents a viable solution to the existing challenges in determining N-Nitrosodiethanolamine (NDELA) in complex matrices. However, it is essential to perform a method validation to ensure the reliability and accuracy of the results. Therefore, HILIC offers a suitable alternative for the detection of NDELA, provided that the method validation is carried out with due diligence.

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Declarations

Conflict of interest It is declared that we do not have any conflicts of interest regarding the present research.

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