The Retention of Some Open-Chain Diamines on a Strong Cation-Exchange Resin in Ion Chromatography

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Abstract

This work reports the results of a study of the influence of the structure of some open-chain diamines on their retention in high-performance ion chromatography (HPIC). We examine a set of eight diamines, in which the *N* atoms are separated by two *C* atoms and are differently *N*- and *C*-alkyl substituted; four of these diamines have the same formula weight. In the other two sets, the diamines are different from each other, either in terms of the length of the alkyl chain between the *N* atoms or in terms of the length of the substituent(s) on the nitrogen atom(s). The mobile phase is a mixture of $HClO_4$ 0.100 mol/L, 0.160 < C_{NaClO_4} < 1.50 mol/L, and 0 < acetonitrile% ≤ 15 (v/v). The amines are detected by integrated amperometry. Interpretation of experimental data shows that (*a*) elution occurs for both ionic and hydrophobic interactions and is dependent on all the components of the mobile phase, (*b*) the diamines are eluted in any case as dications, and (*c*) steric hindrance significantly influences elution times. Some examples of separation are proposed.

Introduction

Amines are in widespread industrial use in dyes and pharmaceutical and biomedical products. They are also present in numerous natural environments, both animal and vegetable. Knowledge of the bioamine concentrations in biological fluids and tissues is of special importance because high concentrations can signal serious disease (1,2). The presence of bioamines in meat and particularly in seafood products is an indicator of freshness and the state of preservation (3). Histamine, even in minimal concentrations, can provoke clinical symptoms similar to those associated with anaphylactic shock. Bioamines are formed during the fermentation of forage held in silos (4). It is evident from this that it is extremely important to be able to optimize amine separations to achieve accurate quantitative determination.

Among the various instrumental techniques available for determining microquantities of amines, high-performance liquid chromatography (HPLC) is the most versatile, especially when used in conjunction with an amperometric (5–10) rather than a conductometric (11–13) or spectrophotometric (14,15) detector. Indeed, the instrumental configuration, which includes an amperometric detector, is used in a large number of laboratories that specialize in food analysis and guarantees the highest standards of analysis of even the most complex environments. As we have already pointed out (16,17), many papers on the HPLC determination of the amine content in various matrices fail to properly justify their choice of instruments of analysis (chromatographic columns, eluents, detectors), and their analytical strategies would appear to be largely dependent on chance. It is for this reason that we have set up a research project whose aim is to study the retention behavior of amines in high-performance ion chromatography (HPIC) as a function of mobile phase composition. This project involves the elution of amines on a column equipped with a strong cationic exchanger with an acidic mobile phase containing different concentrations of inorganic salts, to which acetonitrile is also added. Amperometric detection is carried out by means of integrated square-wave detection (ISWD) (5,6). We have already studied the behavior of NH_2-$(CH_2)_n$-NH_2 (n = 2–10)-type diamines (16) and CH_3-$(CH_2)_{n-1}$-NH_2 (n = 1–6)-type monoamines (17). To continue with this project, we more recently studied the chromatographic behavior of some *N*-alkyl-substituted open-chain diamines (Table I), with the aim of highlighting the influence of structure on their retention and promoting advanced procedures for the polyamines analysis. The amines were divided into homogeneous groups. The first includes eight diamines (D1–D8) in which the *N* atoms are separated from each other by two *C* atoms and are differently *N*- and *C*-alkyl substituted. From this group, we derived two subgroups: the first made up of diamines (D1–D4) with the same molecular weight and the second (D2, D3, and D5–D8) including ethylenediamine and all its *N*-methyl substituted derivatives. We also studied the effect of the length of the methylenic chain between the two nitrogen atoms (D2 and D9) and the length of the nitrogen substituents (D9 and D10).

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Experimental

Reagents

Standard stock solutions of amines were prepared by dissolving the appropriate amount of their purified hydrochlorides in water. $HClO_4$ solution was prepared from corresponding concentrates and standardized against Na_2CO_3. $NaClO_4$ solution was prepared from corresponding solid product that had previously been vacuum hand dried. NaOH was prepared by diluting standard 10 mol/L. HPLC-grade acetonitrile (ACN) was used. All the amines and reagents were Fluka or Aldrich (Sigma-Aldrich Fine Chemicals, St. Louis, MO) products. All solutions were prepared using grade A glassware and ultrapure water (conductivity < 0.1 µS), filtered on a Millipore 0.45 µm (Pall Corporation, Ann Arbor, MI), and then ultrasonicated for 20 min.

Instrumentation

The choice of both the instrumental configuration and mobile phase composition has already been discussed (16,17). The chromatographic system was a DX500 ion chromatographic analyzer (Dionex, Sunnyvale, CA) incorporating a gradient pump and equipped with a thin-layer Dionex ED40 amperometric cell; potential was applied according to the ISWD rules (6). The post-column addition of NaOH was carried out using a Dionex PC10 pneumatic controller. The system was controlled by Dionex Peaknet 4.10 chromatographic software. The column used was the Dionex 4- × 250-mm i.d. CS10 IonPac, with a 4- × 50-mm i.d. CG10 IonPac as guard-column. This strong cationic resin is characterized by uncharged sites between the sulphonic groups so that a polyvalent ion can preferentially coordinate the resin at only one point. Mixtures of 0.100 mol/L $HClO_4$; 0.300, 0.600, 1.00, and 1.50 mol/L $NaClO_4$; and, for each of them, 0%, 3%, 6%, 9%, 12%, and 15% ACN (v/v) were used as eluents.

Results and Discussion

D1–D8 diamines

This group includes two subgroups. The first is composed of four diamines (D1–D4) having the same molecular formula $C_4H_{12}N_2$ [formula weight (FW) = 88.1); D1–D3 are *N*- or *N,N'*-alkyl substituted, and D4 is *C*-dimethyl substituted. The second includes six diamines (D2, D3, and D5–D8) derived from the gradual *N*-methyl substitution in the ethylenediamine; D2 and D3 are isomers. We eluted all these amines with mobile phases as already reported and for each analysis calculated the retention factors, k' [$k' = (t_R - t_0)/t_0$, where t_R = retention time and t_0 = hold-up time = 1.52 min in our experimental conditions]. We also considered that because both Na^+ and H^+ interact with the resin in the same manner, the total cation concentration C_E ($C_E = C_{Na} + C_H$) in the eluent is equal to C_{Na} + 0.100 mol/L.

The amines D1–D4 have the greater differences in k', especially in the elutions carried out with the mobile phase having both the lowest ionic and ACN concentrations (with the eluent in which ACN = 0% and pE = 0.398, we have k' = 42.64, 27.54, 21.09, and 17.78, respectively), and these values gradually level off with increases in both C_{Na} and ACN percentage (with the eluent in which ACN = 15% and pE = –0.204, k' values become 0.69, 0.46, 0.33, and 0.55, respectively). The differences in k' observed for the amines eluted with the same eluents, not linked to the FW, depend on their structure. A comparison of the k' values of diamines D2, D3, and D5–D8 eluted with the same mobile phase concentrations reveals that: (*a*) D5 has the lowest k' value and D2 the highest (an example, for the eluent containing 3% ACN and pE = 0.155, k' = 5.16 and 2.72 for D2 and D5, respectively), according to the structural complexity of the molecules in the series. (*b*) The increasing percentages of ACN in the mobile phase provoke a general reduction in k' values, and the major relative variations in k' (approximately 50% of the total) occur when the ACN in the mobile phase changes from 0% to 3%. The reduction in k' depends on the amine; thus, in the softer elution conditions (pE = 0.398, ACN = 0 %), k' follows the order D5 < D6 < D3 < D8 < D7 < D2 (k' varies from 10.57 to 27.54). But when ACN becomes 15% (pE = 0.398), the k' series is D5 < D8 < D3< D6 < D7 < D2 (k' varies from 8.80 to 13.57). Moreover, if we consider the relative k' variations, we can see that D5 has the smaller reduction (–17%) and D8 the greater (–62%). These observations indicate the diversity of factors contributing to retention, and in the following sections the factors that influence the k' values will be discussed.

The k'–hydrophobicity relationship

The most meaningful nonionic interaction affecting k' values is attributable to the different hydrophobicities of the analyte–eluent systems. Whilst mobile phase hydrophobicity can be easily correlated with its composition, to evaluate amine hydrophobicity it is necessary to identify a reference parameter. For this purpose, we used the partition coefficient P of a certain substance between two immiscible solvents, such as octanol and water. Log P values can be calculated using the Logkow computer program (18), which estimates log P values using a method that adds together the hydrophobic contributions of the various fragments of a molecule. For diamines D1–D8, log P values were –0.66, –0.69, –0.94, –0.75, –1.62, –1.15, –0.48, and –0.26, respectively. Although log P values are estimated statistically and refer to neutral amines, whereas we are dealing with diprotonated amines, it is neverthe-

Table I. Amines Considered in this Paper

Formula	Name	Symbol
NH_2-$(CH_2)_2$-NH-C_2H_5	*N*-ethylethylenediamine	D1
CH_3-NH-$(CH_2)_2$-NH-CH_3	*N,N'* dimethylethylenediamine	D2
NH_2-$(CH_2)_2$-N$(CH_3)_2$	*N,N'* dimethylethylenediamine	D3
NH_2-(CH_2)-C$(CH_3)_2$-NH_2	1,2-Diamino-2-methylpropane	D4
NH_2-$(CH_2)_2$-NH_2	Ethylenediamine	D5
NH_2-$(CH_2)_2$-NH-CH_3	*N*-methylethylenediamine	D6
CH_3-NH-$(CH_2)_2$-N$(CH_3)_2$	*N,N,N'*-trimethylethylenediamine	D7
$(CH_3)_2$N-$(CH^2)^2$-N$(CH_3)_2$	*N,N,N',N'*-tetramethylethylenediamine	D8
CH_3-NH-$(CH_2)_3$-NH-CH_3	*N,N'*-dimethyl-1,3-diaminiopropane	D9
CH_5-NH-$(CH_2)_3$-NH-C_2H_5	N,*N'*-diethyl-1,3-diaminopropane	D10

less possible to interpret some k'–log P relationships. Figure 1 shows k' values for D2 and D5–D8 (let us remember that they have increasing degrees of *N*-methyl substitution starting from D5 = ethylenediamine) versus corresponding log P values (the most positive log P values are indicative of greater hydrophobicity) for three different ACN percentages in the mobile phase. Of all the amines, D5 is the least hydrophobic and, thus, has the lowest k' value. *N*-monomethyl-substituted D6 is more hydrophobic (the estimated methyl contribution to hydrophobicity is not compensated by the estimated increase in hydrophilicity because of the substitution of $–NH_2$ with –NH–), and this is linked to the analyte spending less time in the mobile phase. We reach a similar conclusion when we compare D6 and D2 (*N*,*N*'-dimethyl substituted). D7 (*N*,*N*,*N*'-trimethyl substituted) is more hydrophobic than D2, as D8 (*N*,*N*,*N*',*N*'-tetramethyl substituted) is more so than D7. Therefore, they should each have ever increasing k'; on the other hand, the k' of D7 is lower than that of D2 and that of D8 lower than that of D7. We believe that this anomalous behavior of log k' versus log P for D7 and D8 because of the steric hindrance caused by the further *N*-alkyl substitution in D7 and D8 on the nitrogen, which limits interactions

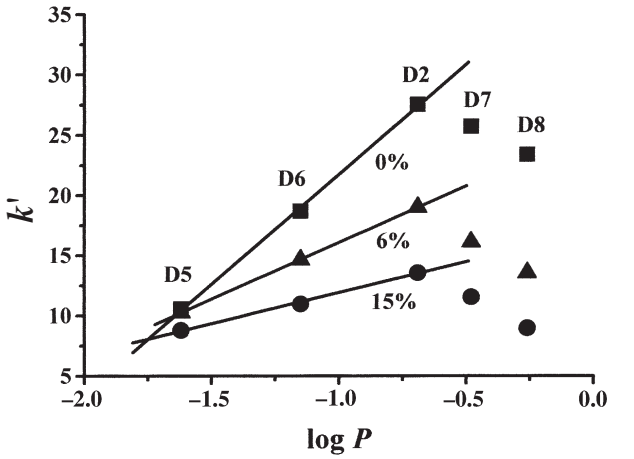

Figure 1. k' v. log P for D2 and D5–8: eluent, $NaClO_4$ = 0.300 mol/L; $HClO_4$ = 0.100 mol/L; and ACN = 0%, 6%, and 15%.

Table II. Parameter According to Equations 4–7

	Slope		Slope	z*
Amine	Equation 4*	Equation 5	Equation 6*	Equation 7
D1	2.33 ± 0.02	–	–0.025 ± 0.001	0.65 ± 0.02
D2	2.42 ± 0.02	2.45	–0.0222 ± 0.0008	0.45 ± 0.01
D3	2.45 ± 0.04	2.45	–0.0210 ± 0.0007	0.30 ± 0.01
D4	2.08 ± 0.02	–	–0.0175 ± 0.0006	0.40 ± 0.01
D5	2.28 ± 0.02	2.28	–0.0075 + 0.0009	0.11 ± 0.01
D6	2.36 ± 0.02	2.36	–0.0169 ± 0.0005	0.32 ± 0.01
D7	2.52 ± 0.04	2.53	–0.028 ± 0.001	0.39 ± 0.01
D8	2.63 ± 0.03	2.62	–0.034 ± 0.001	0.31 ± 0.02
D9	2.40 ± 0.04	2.45	–0.028 ± 0.002	0.35 ± 0.02
D10	2.31 ± 0.06	–	0.043–0.004	0.82 ± 0.04

* ± 3 times SD.

with the sulphonate in the resin and makes retention times for D7 and D8 lower than that for D2. This state of affairs can be described by the linear equations:

ACN 0%: $k' = 40.0 (\pm 0.4) + 18.2 (\pm 0.3) \log P$
($r = 0.9995$) Eq. 1

ACN 6%: $k' = 25.7 (\pm 0.2) + 9.7 (\pm 0.3) \log P$
($r = 0.9999$) Eq. 2

ACN 15%: $k' = 17.1 (\pm 0.3) + 5.1 (\pm 0.3) \log P$
($r = 0.9983$) Eq. 3

[in this paper errors in parameters are reported in parentheses as ± 3 times standard deviation (SD)] calculated from the k' dependence of D5, D6, and D2 on log P. The behavior of D7 and D8 is anomalous, as previously indicated.

The log k'–pE relationship

The k' values obtained for all the amines indicate their strong dependence on cationic concentration [pE = –log(C_{Na} + 0.100)] and on ACN% in the eluent. Going from pE = 0.398 to pE = –0.204 ($0.300 \leq C_{Na} \leq 1.50$ mol/L), a decrease in k' of approximately 95% was observed. To evaluate the influence of the ionic contribution to the chromatographic process (i.e., the log k'–pE relationship), we can use the linear equation (19):

$$\log k' = (y/x)pE + \log I \quad \text{Eq. 4}$$

where x and y = effective charge of the eluent and analyte, respectively, and I = isocratic constant.

In order to calculate slope (y/x) and intercept (log I) values for each diamine, we analyzed the experimental data using the computer program LIANA (20). The results show that (*a*) for each diamine, the slope value (Table II, first column) is the same regardless of the ACN% in the mobile phase, whereas the corresponding intercept values (log I) do depend on ACN% (see also next section); (*b*) all the calculated slope values are higher than two, whereas the theoretical value for a divalent analyte must be equal to two; and (*c*) the differences between theoretical and calculated slope values increase as a function of the number n of methyl substituents. In order to quantitate this last consideration, we analyzed the homogeneous series D2, D3, and D5–D8 and rewrote equation 4 with a new parameter function of n and $y/x = 2$. From LIANA calculations we generated the following:

$$\log k' = [2 + 0.28 (\pm 0.01) + 0.084 (\pm 0.008)n]pE + \log I \quad \text{Eq. 5}$$

The slope values calculated using equation 5 (Table II, second column) are in good agreement with those calculated using equation 4.

The log k'–ACN percentage relationship

As already mentioned in the previous section, the k' values of each amine are differently dependent on the ACN content in the eluent mixture. As an example, D1 eluted with a mobile phase in which pE = 0.398 has k' = 42.64 in the absence of ACN and k' = 17.91 when ACN = 15%, with Dk' = 24.73; whereas D5, in the

same experimental conditions, has Dk' = 1.77. The relationship between elution speed (expressed as log k') and percentage of ACN in the mixture for each diamine and for the same pE value is linear (data processed with LIANA):

$$\text{Log } k' = a \times (\%ACN) + b \qquad \text{Eq. 6}$$

The slope values of each amine, a, are reported in Table II (third column) and indicate log k' variation as a function of the same variation in ACN percentage in the eluent. Parameter b corresponds to the log k' value when ACN% = 0, and the values recalculated using equation 6 reproduce those measured within ± 0.02 units. For amines D1–D4 (same FW), the values of a become less negative going from D1 to D4, indicating a lesser dependence of k' on ACN% in the series. In the subgroup D2, D3, and D5–D8 the *N*-tetramethyl substituted D8 (the most hydrophobic amine) is the "most sensitive" to additions of ACN (has the greatest absolute slope value), whereas the unsubstituted D5 (the less hydrophobic amine) is the "least sensitive" and has the smallest absolute slope value. Figure 2 illustrates this point. The aminic groups, which from D5–D8 switch from primary (the most hydrophilic) to tertiary, also contribute to variation in hydrophobicity. We also observed that the slope values reported in Table II relative to the homologous diamines D2, D3, and D5–D8 are linearly dependent on the number n of methyl groups present in the molecules according to the equation slope = –0.0088 – 0.0064n (with r = 0.9934 and SD = 0.0011). A literature report (21) observed that the retention of diamines eluted on polycarboxylic acid-type cation-exchange resin with nitric acid was only slightly affected by the addition of ACN to the eluent, whereas when we used the CS10 IonPac column (Dionex, Sunnyvale, CA) we observed a significant decrease in retention time even when small amounts of %ACN were added.

The addition of ACN in the mobile phase reduces the dielectric constant of the eluent, and this allows the amines (especially the more hydrophobic ones) to spend more time in the mobile phase rather than interact with the sulphonate in the resin. Consequently, the retention times of the amines, which are heavily influenced by the percentage of ACN in the mobile phase, are lower than those obtained with mobile phases from which ACN is absent.

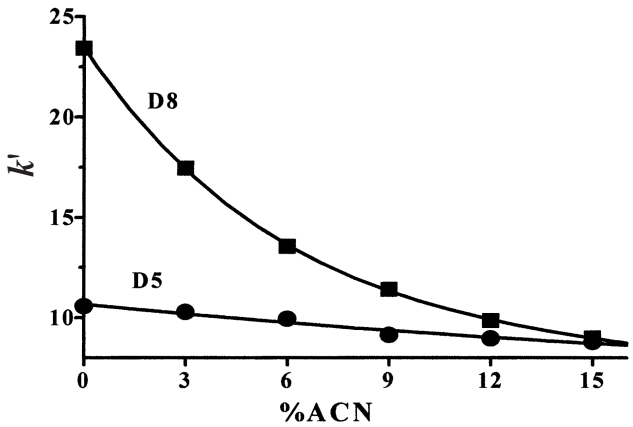

Figure 2. k' v. %ACN: C_{Na} = 0.600 mol/L.

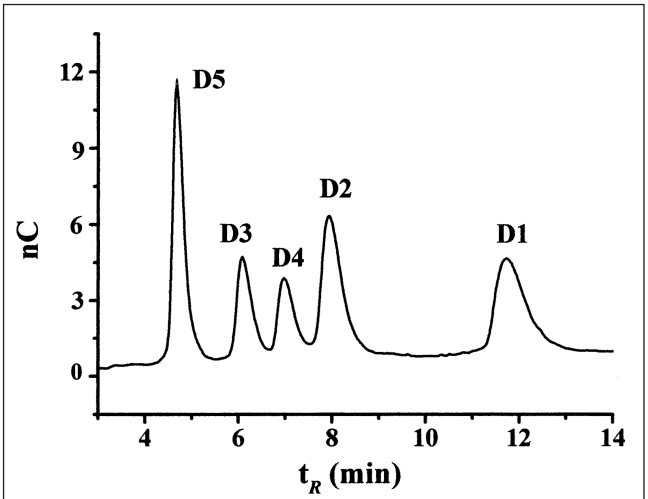

Figure 3. Isocratic separation (0.5000 mol/L $NaClO_4$, 0.100 mol/L $HClO_4$, and 0% ACN) of D1–D5 mixture. Amine concentrations = 5 µmol/L.

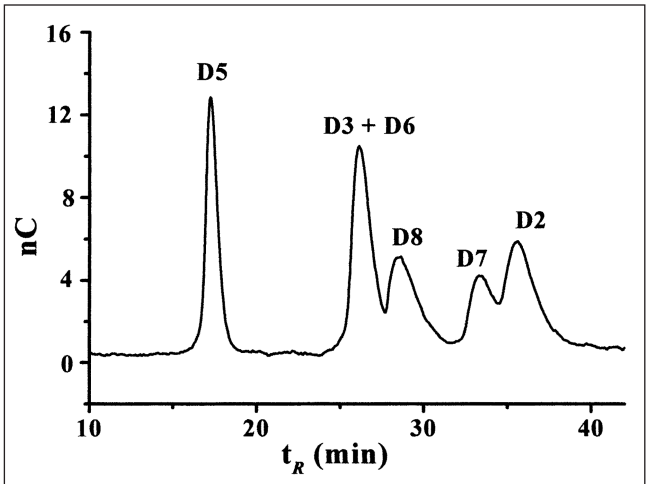

Figure 4. Isocratic separation (0.300 mol/L $NaClO_4$, 0.100 mol/L $HClO_4$, and 3% ACN) of D2, D3, and D5–8 mixture. Amine concentrations 5 µmol/L.

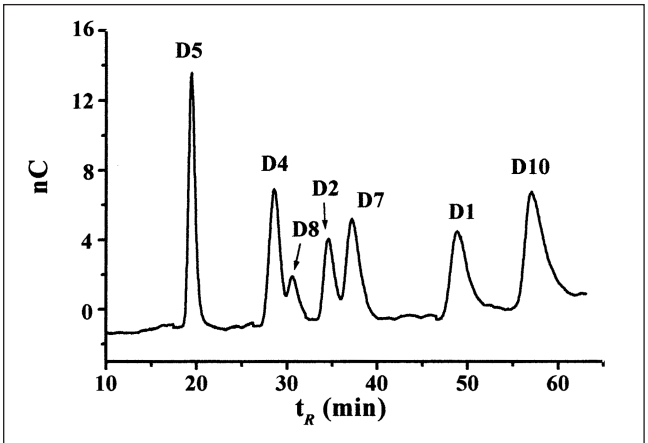

Figure 5. Gradient separation (0.300 mol/L $NaClO_4$, 0.100 mol/L $HClO_4$, and ACN from 3–9% from 0–60 min) of mixture D5, D4, D8, D7, D2, D1, and D10. Amine concentration 5 µmol/L.

The log k'–ACN%–pE relationship

With the aim of obtaining an equation to describe the dependence of k' on the ionic (pE) and on ACN%, we combined equations 4 and 6:

$$\log k' = z + 0.5[(y/x) \times pE + a \times (\%ACN)] \quad \text{Eq. 7}$$

in which $z = (b + \log I)/2$. This equation was tested using LIANA to recalculate parameters z, y/x, and a. The values of y/x and a are exactly the same (± 1% as the mean) as those reported in Table II, and z values (also reported in Table II, last column) are very close to those calculated by (b + log I)/2. The consistency of the results, along with the low SD values, is a good indication of the validity of the proposed equations.

N,N'-substituted diaminopropane

Here, we have two diamines D9 and D10 (*N,N'* dimethyl and *N,N'* diethyl substituted, respectively) with a propylic chain between the nitrogen atoms. The k' values of D9 are comparable to those of the other diamines, whereas those of D10 are approximately doubled, especially when the mobile phases have low salt concentrations. The k' value of D10 for pE = 0.398 and ACN% = 0 exceeds 1 h. For these diamines too, an increase in ACN percentage in the mobile phase tends to level out k' values. Chromatographic behavior is consistent with the marked increase in hydrophobicity from D9 (log P = –0.20) to D10 (log P = 0.79). To establish the value of the y/x relationship (equation 4), we processed experimental data using LIANA. The slope values (see Table II, first column) confirm that the analyzed amines interact with the sulphonate as diprotonated amines. The slope value for D9 is comparable both with that of D2 and the value recalculated using equation 5. The D10 slope, however, is significantly different. In order to evaluate the influence of the length of the intra-aminic chain on elution speed, we compared the k' values of D2 and D9. Both the hydrophobic contribution [log P(D2) = –0.69; log P(D9) = –0.20] and molecular weight indicate D2 to be faster than D9. In reality, the opposite occurs (with all the eluents used), probably because the distance between the two positive charges in D9 is different from that between the negative charges in the sulphonate groups of the resin in the column, and this promotes weaker interactions. Also significant is the high Δ value [Δ = difference in intercept values (equation 4) between elutions carried out with mobile phases containing 0% or 15% ACN] of D10 = 0.66, which is consistent with its high degree of hydrophobicity because of the two *N*-ethyl substitutions. By comparison, Δ values for D5 (which has the lowest degree of hydrophobicity of all the diamines examined here) is 0.10.

Separations

We now report some examples of separations. An analysis of amines D1–D5 (Figure 3) is straightforward. The separation is complete in little more than 12 min from the introduction of the sample, whereas effective separation of the amines is complete in less than 7 min. For the series D2, D3, and D5–D8 (Figure 4), it was not possible to separate amines D3 and D6 because they were coeluted in all experimental conditions. A mobile phase with C_{Na} < 0.300 mol/L was not tested because the overall analysis time increased considerably. Total analysis time was under 40 min, and net analysis time for amines was approximately 24 min. Except for the above-mentioned D3–D6 pair, the separations were sufficient to allow quantitative determination. Finally, Figure 5 reports an example of gradient separation of a more complex mixture, also containing D10. In this case, the net analysis time was approximately 40 min. As regards the detection limits for the analytes under investigation, with ISWD, the estimated detection limit is certainly below 0.1 µmol/L.

Conclusion

The main conclusions of the paper confirm that the two main mechanisms influencing the retention behavior of the analytes studied depend on (*a*) the dynamic and reversible ionic interaction equilibrium between the protonated amine and the sulphonate anion in the resin, modulated by the C_{Na} in the mobile phase (Na^+also forms ion pairs with sulphonic polyanions) (22) and (*b*) the dynamic and reversible amine distribution equilibrium between resin and mobile phase, linked to the different hydrophobicity of analyte–eluent systems and modulated by the %ACN present in the mobile phase. We have proposed linear equations 4 and 5 to describe the dependence of k' on C_{Na} (ionic effect) and equation 6 to describe the dependence of k' on the %ACN (hydrophobic effect) in the mobile phase, whose parameters were calculated. Equation 7, which summarizes equations 4 and 6, confirms the consistency of the results obtained. A mechanism for reversed-phase ion-pair liquid chromatography was already proposed (23). In this investigation, the negative charges were temporarily created on a C_{18} column by adsorbed Na-alkylsulphonates, whilst in this work the sulphonate anions were always present as a functional group. Moreover, Bidlingmeyer et al. (23) report that a pair of ions (not necessarily an ion pair) had been adsorbed onto the stationary phase. In our case, we observed the formation of an ion pair of significant strength, as reported in a previous work (22) on the sequestering ability of some sulphonic polyanions towards protonated polyamines and alkali metal cations.

The steric hindrance observed in the amine–resin interaction, caused by *N*-methyl substitution(s), perhaps also makes a significant difference to elution speed. We have shown that D7 and D8 (tri- and tetra-methyl substituted) have lower k' values than D2 (di-methyl substituted), even if they are more hydrophobic (Figure 1). The D9–D10 pair confirms the role of the hydrophobic effect on elution speed. The higher k' value of D2 than of D9 seemed illogical (D9 is more hydrophobic than D2); the greater distance between the nitrogen atoms in D9 probably causes less interaction with the negative charges on the sulphonate. As concerns the influence of the FW diamines on their elution speed, we were not able to generate a clear picture of this. The great differences in k' values in the D1–D4 series (same FW) seem to suggest that FW does not have a significant influence.

Acknowledgments

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