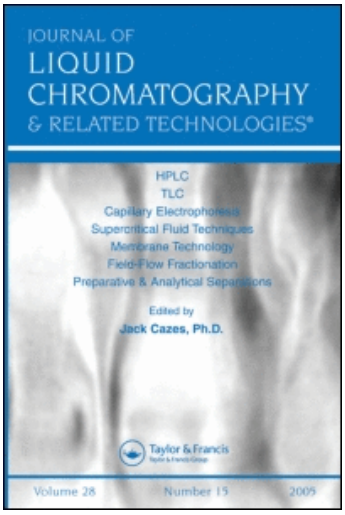

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Borai, Emad. H. , Lasheen, Y. F. , Seliman, A. F. and Aly, H. F.(2008) 'Gradient Elution Method for Successive Separation of Common Cations and Hydrophobic Amines using Suppressed Ion Chromatography', Journal of Liquid Chromatography & Related Technologies, 31: 6, 838 — 849
To link to this Article: DOI: 10.1080/10826070801891460
URL: http://dx.doi.org/10.1080/10826070801891460

Journal of Liquid Chromatography & Related Technologies®, 31: 838–849, 2008

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070801891460

Gradient Elution Method for Successive Separation of Common Cations and Hydrophobic Amines using Suppressed Ion Chromatography

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Abstract: In mixed waste, the separation and sequential determination of alkali and alkaline earth metals mixed with hydrophobic amines represent a challenging analytical problem. The effect of a new mobile phase (amido-sulfonic acid, ASA) on the suppressed ion chromatographic separation of alkali and alkaline earth metals and hydrophobic amines on CS12A analytical column was investigated. The addition of surface modifier to the eluent appears to provide better interfacial compatibility between the mobile and stationary phase and facilitates the rapid equilibration of analytes. Incorporation of a very low concentration of the additive may also alter the stationary phase surface by creating a fine tuning and improves the partition characteristics of the analytes. A dramatic and sufficient elution capability of amido-sulfonic acid (ASA) for sequential separation of the analytes was reported and rational mechanisms for the separated analytes are proposed. ASA can act as an ion pairing agent resulting in the separation of a wide variety of amines. The new mobile phase (ASA) is proven to have more successful separation over methansulfonic acid (MSA), even with eluent free solvent. The proposed method shows that a profound particular effect on the separation of aliphatic diamine (Ethylenediamine) and organic amine (Cyclohexylamine) was achieved in addition to all common cations and amines using isocratic elution of 18 mM of ASA without organic eluent modifier.

Keywords: Ion chromatographic separation, Amines, Alkali and alkaline earth metals, Methansulfonic acid (MSA), Amidosulfonic acid (ASA)

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INTRODUCTION

In ion chromatography, the eluent composition provides the main flexibility for manipulating the separation and detection of various analyte ions.[1] The eluent components that could be used in ion chromatography with suppressed conductivity detection must be such that they are removed from the eluent flow and/or converted to weakly conducting species during the suppression process.

It should not cause additional peaks as a product of the suppression reaction. The net effect of the suppression process is to reduce the background conductance of the eluent and to enhance, simultaneously, the detection of the analyte ions.[2,3] Selection of an eluent is thus crucial to obtain efficient separation and detection.[4] The specifications that apply to a substance to be used as eluent in suppressed chromatography are limited.[5]

The term amines encompasses a wide variety of compounds involved monovalent, divalent, polyvalent, hydrophobic, or hydrophilic amines. These amines have wide uses in various nuclear and non-nuclear applications. Some amines are used in the power for industry cooling waters, where as others are used as chelating and complexing agents for radioactive waste treatments. Due to their charge, some amines have very strong cation exchange interaction with the cation exchange groups in the stationary phase. Very high acid concentrations are required to elute them effectively from a high capacity carboxylated cation exchange column.

In many applications involving amines,[6,7] due to either solubility of the analyte or because the amine is hydrophobic and, therefore, can strongly interact by adsorption with the polymeric stationary phase, it is necessary to add solvent to the eluent.[8,9] In the case when the analytes require solvent to keep them in solution, or when they are more hydrophobic, the high cross-linking of the substrate polymeric bead allows the column to be used with an eluent containing solvent, especially for the separation of various organic amines and aliphatic diamines. Ye et al.[10] reported the use of alkylsulfonic acid additives to separate underivatized amino acids. Clearly, the concept of mobile phase additives simply masking interactions with the underlying chromatographic support does not explain the observed effects.[11–13] McCormick and Karger[14] found that as little as 2% (v/v) of an organic modifier of higher molecular mass added to a methanol–water or acetonitrile–water mobile phase can have a major effect on HPLC separations. Tanaka et al.[15] obtained a broad, much tailed peak for triethanolamine when water was used as the mobile phase. However, an aqueous mobile phase containing 0.2 M xylitol, fructose, glucose, or sorbitol gave a sharp, well resolved peak for each of the four analytes. The improved behavior shown was due to the increased hydrophilicity of the surface resulting from adsorption of the sugar.

In many applications of this nature with the CS12A, it is possible to use the column under elevated temperature conditions instead of adding solvent to the eluent. Separation of the highly retained aliphatic diamines was accomplished by raising the temperature to 40°C and using a sulfuric

acid-acetonitrile gradient.[16] The longer the carbon chain length of the diamine, the more hydrophobic it is, and the longer it is retained in the column substrate through reversed phase adsorption. Temperature has the added potential benefit of aiding separations that are difficult, both through an increase in peak efficiency, as well as changes in analyte selectivity. In some cases, the use of solvent in the eluent can be avoided by increasing the column temperature, thus reducing operating cost and environmental impact of toxic solvent.

In this study, we actually investigated two different eluents such as methansulfonic acid (MSA) and amidosulfonic acid (ASA) for separation of alkali metals (Li, Na, K, and Cs), alkaline earth metals (Mg, Ca, Sr, and Ba), aliphatic amines (Ethanolamine, Diethylamine, Ter-butylamine, Triethylamine Ethylenediamine), and organic amines (Cyclohexylamine), with or without acetonitrile as organic modifier. The compatibility of the new eluent (ASA) in suppressed chromatography is tested in detail.

EXPERIMENTAL

Chemicals and Reagents

All chemicals used were of analytical purity grade. Stock solutions of methansulfonic acid (MSA) (Fluka, Ronkonkoma, NY, USA) and amidosulfonic acid (ASA) eluents were prepared by dissolving a known volume and weight, respectively, of each compound in bidistilled water to give 100 mM. Lower eluent concentrations were prepared by proper dilution of related stock solutions. Acetonitile was obtained from Allied chemical (Morristown NJ, USA). Sodium chloride, potassium chloride, magnesium metal, cesium chloride, strontium chloride, calcium chloride, lithium chloride, and barium chloride were purchased from BDH (England). Diethyl amine, triethyle amine, and cyclohexyleamine were from Merck (Germany). Other chemicals were obtained from different suppliers including, ethylenediamine and terbutyl amine from WINLAB (England) and ethanolamine from Bikio (India). Standard stock solutions were quantitatively prepared by dissolving requisite amounts of each compound, or its salt, in double distilled deionized water. All measurements were carried out at room temperature using a 50 μL injection loop.

Instrumentation

An ion chromatographic (IC) system from Dionex Corporation, Sunnyvale, CA., USA, model 2000i/sp is used. It consists of a gradient pump GPM-2 to adjust the eluent flow rate during all the chromatographic separations and a conductivity detector, model CDM-3 combined with a cation self regenerating suppressor (CSRS). The 250 X 4 mm I.D. IonPac CS12A analytical

column used is coupled with a CG12A guard column. The analytical column and related guard are packed with divinylbenzene/styrene resin functionalized with relatively weak phosphonic and carboxylic acid radicals, with high selectivity for hydronium ions. The weak carboxylate functional groups need to use eluents with low ionic strength to isocratically elute both monovalent and divalent cations in relatively short periods of time with both cation exchange and reverse phase properties. CS12A columns are also compatible with organic solvents including 100% acetonitrile, 20% tetrahydrofuran, or 100% aqueous eluents, without losses in column performance. Data collection and operation of the whole system are controlled by Dionex AI-450 chromatography software via an ACI-2 advanced computer interface. The output of the conductivity detector is automatically normalized so that readout of 1 μS is equivalent to 1 μS/cm. The background conductivity in all cases was below 0.2 μS, and typical noise level was about 0.2 nS.

RESULTS AND DISCUSSION

Effect of Eluent Concentration

All the analytes were arranged according to their retention times and elution order. As shown in Table 1, the retention time generally decreases as the concentration of eluent in aqueous mobile phase increases in both MSA and

Table 1. Effect of MSA and ASA concentration on the retention time of the investigated analytes

	Eluent concentration, mM							
	MSA, mM				ASA, mM			
Analyte	10	15	20	30	10	15	20	30
Li	5.93	4.48	3.37	3.18	5.53	4.27	3.83	3.47
Na	7.37	5.30	3.98	3.62	8.82	5.10	4.35	3.97
Ethanolamine	8.23	6.20	4.65	4.18	8.28	6.17	5.08	4.55
K	11.07	7.78	5.52	4.78	10.13	7.23	6.03	5.28
Diethylamine	13.57	9.13	6.22	5.82	14.30	9.40	6.82	6.48
Ter-butylamine	13.97	9.33	6.75	6.48	15.80	10.60	7.33	7.18
Cs	17.16	10.37	7.90	6.48	14.90	10.70	8.88	7.28
Mg	38.17	16.92	9.47	5.70	37.42	17.08	11.33	7.40
Ca	49.63	21.50	11.73	6.73	48.23	21.50	14.08	8.80
Sr	50.91	23.70	13.10	7.32	52.32	24.85	16.1	9.57
Triethylamine	40.20	25.8	15.22	14.42	44.72	28.35	16.40	15.10
Ba	58.43	34.43	18.53	9.80	60.7	35.70	22.52	13.02
Ethylenediamine	100.0	60.62	32.00	15.23	105.0	61.15	34.60	20.92
Cyclohexylamine	120.0	74.8	32.42	30.1	123.1	82.3	37.60	31.50

ASA systems. Using MSA, the retention times of all the analytes are lower than that of the corresponding values using ASA. The data revealed that insufficient separation of most of the investigated analytes was obtained at relatively high concentrations, 30 and 20 mM, of each of MSA and ASA. Compared to the high and low eluent concentration, 30 and 10 mM, respectively, it was found that the sequence of elution order of triethylamine and Ba was changed as a result of eluent dilution of both MSA and ASA systems. In middle eluent concentrations, 15 and 20 mM, similar elution orders were observed in both MSA and ASA systems. This is because changing the eluent strength has a larger effect (power of 2) on divalent cations than on aliphatic and organic amines.

As shown in Figure 1, plotting a logarithmic relationship between the eluent concentration and the selectivity factor (K′) revealed that at low MSA concentration (10 mM), coelution of diethyl amine and ter-butylamine occurred in which they were successfully separated by 10 mM of ASA; however, 10 mM of MSA is better for separation of diethyl amine and Cs than 10 mM of ASA, due to change in elution sequence of ter-butylamine and Cs that leads to peaks overlap. Furthermore, long retention times and fronted peaks of ethylenediamine and cyclohexylamine were obtained at 10 mM of each of the two eluents.

Effect of Eluent Modifier Concentration

The effect of acetonitrile concentration as eluent modifier on the separation efficiency was tested at various percentages ranging from 3.0 to 25%. Eluent concentration of each of MSA and ASA was kept at 20 mM. The mobile phase additive is believed to coat the stationary phase surface by a dynamic equilibrium. The coated surface is more hydrophilic and facilitates the efficient partitioning of analytes between the mobile and stationary phases. Unfortunately, unstable baseline was observed due to the addition of acetonitrile. This base line instability is more effective in ASA than that of MSA. As shown in Table 2, there was insignificant improvement in the retention times of most of the investigated analytes, especially common cations and amines that have lower molecular atomic weight. In both eluent systems, increasing the concentration of acetonitrile from 3.0 to 25% leads to deterioration of the peak performance of all analytes.

Profound effects of acetonitrile as organic modifier to the two eluents on the retention times of triethylamine, ethylenediamine, and cyclohexylamine was obtained with various degrees of magnitude.

Particularly, in the MSA system, increasing the solvent concentration from 3.0 to 5.0% leads to an overlap between Ca and Sr, which are separated well by using ASA. On the other hand, in the case of ASA, lowering the acetonitrile concentration from 5.0 to 3.0% leads to an overlap between Mg and Triethylamine. However, at 20 mM of ASA and 5.0% of acetonitrile, serious overlap between diethyl amine ter-butylamine was observed, which was improved at 3.0% of acetonitrile.

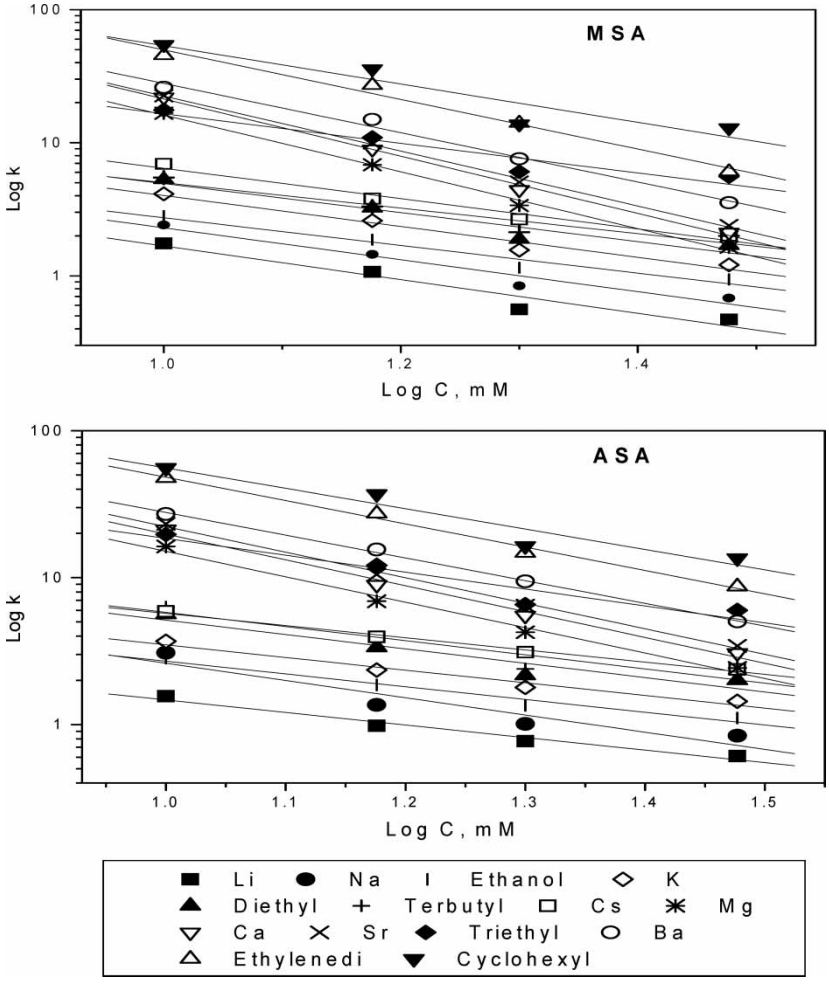

Figure 1. Logarithmic relationship between the eluent concentration and the selectivity factor.

Therefore, it is not recommended to use acetonitrile as organic modifier especially with ASA eluent for separation of common cations and amines which have low molecular weight.

Gradient Elution for Separation of Mixture of Common Cations and Amines

Figure 2, shows an isocreatic elution of mixed analystes using 15.0 mM of MSA without organic modifier at 1.0 mL/min eluent flow rate. The

Table 2. Effect of acetonitrile concentration on the retention time of the investigated analytes

	Acetonitrile concentration (%)							
	MSA, 20 mM				ASA, 20 mM			
Analyte	3%	5%	7%	25%	3%	5%	7%	25%
Li	3.25	3.53	3.17		3.53	3.53	3.55	
Na	3.85	4.18	4.11		4.18	4.13	4.17	
Ethanolamine	4.62	4.32	4.0		4.92	4.75	4.65	
K	5.37	5.82	5.31		5.82	5.68	5.67	
Diethylamine	5.22	4.92	4.9	5.32	5.68	5.22	5.30	6.30
Ter-butylamine	5.55	5.77	5.3		6.00	5.30	4.98	
Cs	7.8	7.3	7.2		8.82	8.35	8.22	
Mg	8.82	10.42	8.7		10.42	10.10	9.85	
Ca	10.87	12.06	10.50		12.95	12.43	12.18	
Sr	14.12	12.6	11.62		15.4	14.2	12.89	
Triethylamine	9.75	7.89	7.10	6.52	10.08	7.93	7.55	7.78
Ba	19.40	18.1	15.88		20.1	19.9	16.2	
Ethylenediamine	27.87	24.37	23.68	19.73	34.20	31.02	30.05	26.78
Cyclohexylamine	17.42	11.07	11.00	7.55	18.00	13.10	11.63	8.63

chromatogram shows good separation efficiency for the first nine analytes, however poor detection sensitivity of triethylamine and Ba. Too long a retention time of ethylenediamine was observed while cyclohexylamine was retained on the analytical column.

To overcome the long elution time of ethylenediamine and cyclohexylamine (organic amine), trials were performed by using mixture of 15 mM of MSA and 3.0% of acetonitrile. As shown in Figure 3, shorter retention

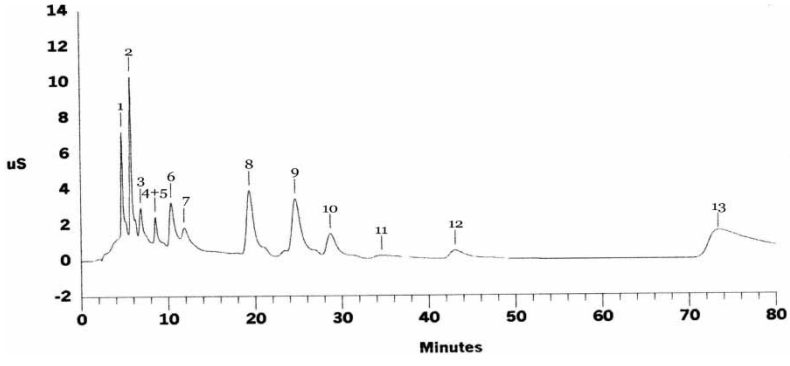

Figure 2. Isocratic elution of mixed analystes using 15.0 mM of MSA.

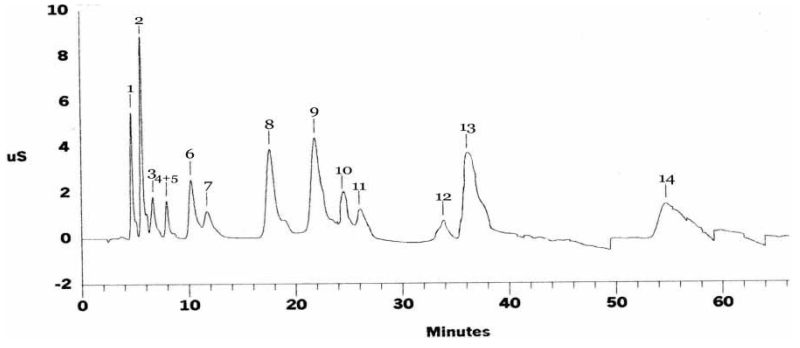

Figure 3. Isocratic elution of mixed analytes using mixture of 15 mM of MSA and 3.0% of acetonitrile.

times of ethylenediamine and cyclohexylamine were obtained. This result suggested that hydrophobic interaction of amines would be the main separation mechanism on ion chromatographic columns containing mixed carboxylic and phosphonic acid groups. The hydrophobic nature of other amines, results in partitioning into the polymeric substrate of previous stationary phases, so that organic solvent is required to elute them effectively. i.e., acid additive (acetonitrile) increased selectivity, presumably due to its ability to alter non-specific adsorption. Furthermore, the detection sensitivity of triethylamine and Ba were improved due to increases in the peaks sharpness.

Attempts to get better separation performance was carried out using gradient time events with various MSA concentrations. Starting from zero to 14.9 min, the eluent concentration was kept at 15 mM. From 15 to 80 min, the eluent concentration was kept at 20 mM of MSA mixed with 3.0% acetonitrile. The chromatogram in Figure 4 shows shorter retention

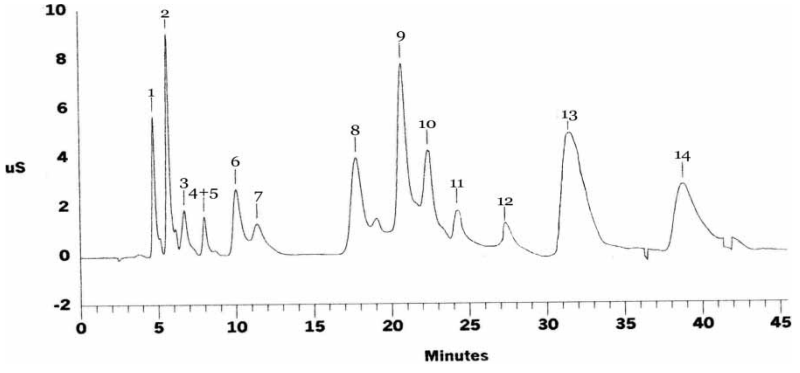

Figure 4. Gradient elution of mixed analytes using: (a) 15 mM of MSA from 0.0 to 14.9 min, (B) 20 mM of MSA mixed with 3.0% of acetonitrile from 15.0 to 50.0 min.

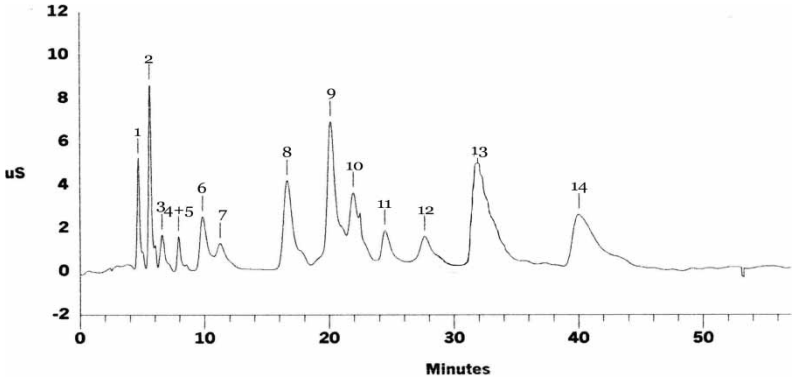

Figure 5. Gradient elution of mixed analytes using: (a) 15 mM of MSA from 0.0 to 14.9 min, (B) 15 mM of MSA mixed with 3.0% of acetonitrile from 15.0 to 80.0 min.

times, particularly for ethylenediamine and cyclohexylamine, while non-baseline separation of Ca, Sr, and triethylamine was observed. Therefore, lowering the eluent concentration to 18.0 mM with 3.0% acetonitrile during the second half of the run was investigated. As shown in Figure 5, the lowering in eluent concentration leads to good base line separation of Ca alone, however, non-baseline separation was still observed between Sr and triethylamine.

Alternative trials using ASA at the same experimental conditions as previously applied with MSA were tested to overcome some of the limitations such as uses of organic solvent as well as the encountered toxicity of MSA itself. Furthermore, based on the preliminary data as shown in Table 1, additional separation efficiency of ASA is expected. In Figure 6, a gradient

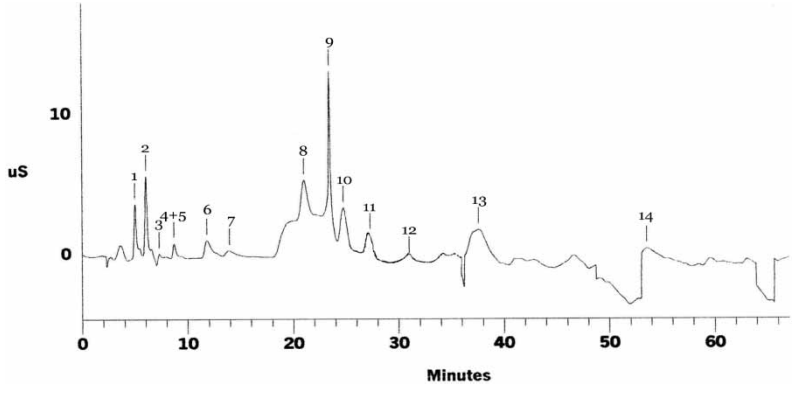

Figure 6. Gradient elution of mixed analytes using: (a) 15 mM of ASA from 0.0 to 14.9 min, (B) 18 mM of ASA mixed with 3.0% of acetonitrile from 15.0 to 80.0 min.

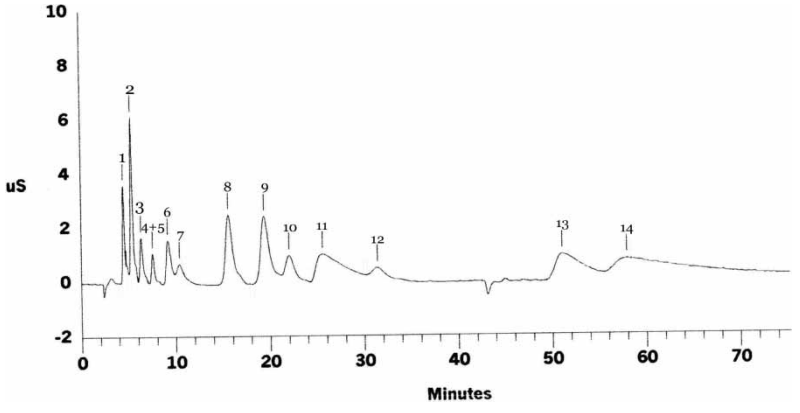

Figure 7. Isocratic elution of mixed analytes using 18 mM of ASA.

elution program was tested. ASA of 15.0 mM was applied from zero to 14.9 min., while 18.0 mM of ASA mixed with 3.0% acetonitrile was used starting from 15.0 min to the end of the run. Fortunately, the addition of 3.0% of acetonitrile demonstrated a high deterioration effect on the base line stability in the case of the ASA eluent as compared with its effect on MSA.

Furthermore, the gradient concentration of ASA from 15 to 18 mM in the presence of acetonitrile presented insufficient separation of Ca, Sr, and triethylamine, as well as the non-uniformed peak of cyclohexylamine. Lowering the percentage of acetonitrile even to 1.0% did not improve the base line stability.

A trial for separation of all the investigated analytes was tested using the isocratic elution procedure with 18.0 mM of ASA without acetonitrile. The chromatogram in Figure 7, shows high separation performance for all the analytes with stable base line throughout the whole run. This method could be used as an alternative method to the mixture of MSA and acetonitrile. The use of ASA has the added benefit of improving the peak asymmetries of the analytes, especially for the Ca, Sr, triethylamine, ethylenediamine, and cyclohexylamine. This enabled the column not only to be used without solvent containing eluent, but also to be applied for sufficient qualitative and quantitative analysis of various samples containing alkali, alkaline earth metals, and hydrophobic amines.

Separation Mechanism

The separation mechanism is considered simply to be the partitioning of the analytes between the predominantly aqueous mobile phase and the stagnant water inside the resin site. However, several authors have proposed a mixed

mode mechanism in which partitioning also occurs between the sample solutes and the polymeric resin matrix.[9,17,18]

Li, S. and Fritz, J.S. stated that, although addition of a low concentration of an alcohol or diol to the mobile phase may shift the stationary mobile phase equilibrium somewhat by stronger solvation of the analytes in the mobile phase, it is difficult to explain the effects noted by solvation alone because the mobile phase was at least 98% water in all cases.[13]

In our work, to summarize the reaction mechanism that controlled the separation efficiency, it could be suggested that the retention elution process first uses the unique selectivity of the carboxylate site along with a mild chelating agent in the eluent that competes with the ion exchange sites for monovalent and divalent ion retention. Second, due to the macroporous substrate polymeric nature of the CS12A column, hydrophobic analytes (amines) can be separated on the column through reversed phase adsorption and based on the differences in their hydrophobicity. Third, ion pair formation of the analytes with the lone pair of electrons either on sulfur atoms and nitrogen atoms of the amido group of ASA, promote the separation efficiency by means of various reaction rates.

CONCLUSIONS

However, several of the analytes could exist as a mixture of their protonated and molecular forms and thereby give significantly broader peaks. The uses of ASA as eluent and the CSI2A analytical column provide higher overall peak efficiencies and improved peak symmetries for both inorganic cations, aliphatic and organic amines. Because the eluent contained no organic solvent, the suppressor can be used in the more convenient autosuppression recycle mode. A simple isocratic elution program provides almost imperceptible baseline change that could alter chromatographic separation and particularly shape selectivity.

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Received March 1, 2007
Accepted September 27, 2007
Manuscript 6076