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Separation of primary and secondary amines as their sulfonamide derivatives by reversed-phase high-performance liquid chromatography

Muhammad Idiris Saleh*, Fen Wei Pok

Analytical Division, School of Chemical Sciences, 11800 Universiti Sains Malaysia, Penang, Malaysia

Abstract

A series of simple and low-molecular-mass aliphatic amines were derivatized with 8-quinolinesulfonyl chloride. Mixtures of the respective substituted sulfonamides were separated by reversed-phase high-performance liquid chromatography (RP-HPLC) and the sulfonamides were found to be highly sensitive to ultraviolet detection. This pre-column derivatization facilitated good separation of eleven amines with the separation factors, α, better than 1.10 and the separation time within 20 min. The optimization of separation was conducted and investigated on intermediate polarity (cyano) and apolar (hexyl) stationary phases. A linear relationship was observed between the retention and the incremental carbon number of sulfonamides formed and thus making possible the retention prediction of the amine derivatives on RP-HPLC columns.

Keywords: Derivatization, LC; Amines; Quinolinesulfonamides

1. Introduction

Chromatographic analysis of low-molecular-mass aliphatic amines has attracted the interest of many researchers [1–4] as these compounds can act as precursors to carcinogenic N-nitroso compounds which lead to a wide variety of tumors in many animals [5]. Aliphatic primary and secondary amines are known to be widely distributed in the environment as they are produced endogenously by living organisms and synthesized in bulk as raw materials for both chemical and manufacturing industries. The problem in the analysis of amines is that they are insensitive towards commonly used detectors such as UV detectors, electrochemical detectors, fluorescence detectors, conductivity detectors, etc. A common approach to the solution of this problem is to derivatize with a chromophoric reagent, the details of which can be found in the literature. Among such methods are the separation of amines following derivatization with high UV-absorbing reagents such as *p*-nitrophenylacetamides [6], trinitrobenzene sulphonate [7] and related nitrophenyls [8], *p*-benzoquinone [3], 1,2-naphthoquinone-4-sulphonate [9] and also some acridinium trifluromethanesulphonates [10]. A series of benzenesulfonamides and benzamides have also been utilized for use in spectrophotometric detection of amines after LC [11]. Apart from this, dabsyl chloride [1,12] and Dns chloride [13–16] have been used in the determination of some amine containing samples. Some reagents have also been evaluated as chiral derivatizing agents in the separation of enantiomeric amines [17–19].

Besides the derivatizing agents cited above, the separation of amine drugs of ephedrine, pseudoephedrine, methamphetamine, phenylpropanolamine,

*Corresponding author.

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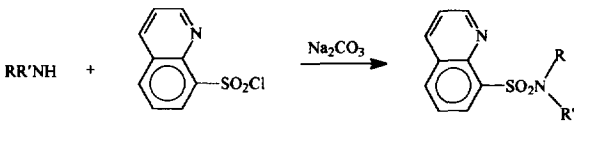
phentermine, phenmetrazine and amphetamine have been achieved by converting amines to sulfonamides, as well as the use of substituted amides to enhance detectability and chromatographic properties [20]. All these analyses of primary and secondary amines were feasible because they react readily with acid chlorides to form substituted amides in basic conditions, as shown in Eq. 1.



I II III

where I = amine
II = 8-quinolinesulfonyl chloride
III = amine 8-quinolinesulfonamide; R = alkyl group
R' = alkyl group or H

Since 8-quinolinesulfonyl chloride is susceptible to nucleophilic attack by amines and also due to the strong UV-absorbing character of the sulfonamides formed, this compound was chosen as the derivatizing agent in our studies for the separation of simple primary and secondary aliphatic amines. The eleven amines successfully separated were namely methylamine, ethylamine, propylamine, butylamine, pentylamine, heptylamine, octylamine, dimethylamine, diethylamine, dipropylamine and dibutylamine.

2. Experimental

2.1. Reagents and chemicals

The amines and 8-quinolinesulfonyl chloride, ≥98% were obtained from Fluka (Buchs, Switzerland). Other solvents and chemicals were HPLC (Ajax Chemicals, NSW, Australia) or analytical-reagent grade purity (BDH, Poole, UK) and were used without purification. Doubly-distilled water was used to prepare the acetate buffer solutions.

2.2. Instruments

Mobile phase was delivered using a Shimadzu LC-6A solvent delivery module (Kyoto, Japan). Eluates were detected at 254 nm by a Hewlett-Packard HP 1050 Series variable-wavelength detector (Waldbronn, Germany) and a Shimadzu Chromatopac C-R6A (Kyoto, Japan) integrator was used for area integration. Samples were loaded with a low-dead-volume Rheodyne Model 7125 injector (Cotati, CA, USA) equipped with a 10-μl loop. Spherisorb Nitrile (10 μm) and Hexyl (10 μm) analytical columns (25 cm×4.6 mm I.D.) from Phase Separations were preceded by a 2 cm×4.6 mm Supelguard column (Supelco, Bellefonte, PA, USA) and used at ambient temperature. Mass spectra were obtained from a Hewlett-Packard HP 5989A MS Engine (Palo Alto, CA, USA) through direct insertion probe. Elemental analysis were carried out on a Control Equipment Corporation CEC Model 240-XA elemental analyzer (Lowell, MA, USA).

2.3. Derivatization procedure

A volume of 0.5 ml standard amines were diluted into 100 ml with 0.5 *M* sodium carbonate. These solutions were then further diluted by a factor of 100. A 10.0-ml solution of 8-quinolinesulfonyl chloride (0.025 g dissolved in 100 ml acetonitrile (MeCN)) was mixed with 2.0 ml amine solution in a test tube. The test tube was sealed and allowed to react for 20 min at 65°C in a water bath [20]. The test tube was cooled and extracted twice with 30 ml chloroform ($CHCl_3$). The organic fraction was then separated and dried with magnesium sulfate, and evaporated to dryness under a stream of nitrogen. The amides were then redissolved in with 1 ml of $CHCl_3$.

2.4. Chromatographic conditions

The mobile phase consisted of methanol (MeOH) or acetonitrile (MeCN) as the organic modifier, and acetate buffer solution in the presence of triethylamine (TEA) as a masking agent. Acetate buffer was prepared by mixing the same volume of 0.01 *M* sodium acetate and 0.01 *M* acetic acid. The amine derivatives were eluted at a flow-rate of 1.5 ml/min, resulting in a pressure of $1.0–1.2\times10^7$ Pa. The mobile phases were vacuum-filtered through 0.45-μm nylon membranes from Supelco and every

mixture was degassed by sonication under vacuum for 10 min prior to its use. No less than thirty column dead volumes were allowed for column equilibration upon a change of mobile phase; where the dead volume of the system was determined as the first baseline disturbance after injection of samples. Separation was performed under isocratic conditions with each capacity factor, k' value reported from the arithmetic mean of triplicate injections.

3. Results and discussion

3.1. Separation of primary and secondary amines

The initial experiments of this study were the synthesis and confirmation of the 8-quinolinesulfonamides of representative amines. Low-molecular-mass primary and secondary amines that are relatively uncrowded around the nitrogen atom reacted in basic medium to give easily characterized substituted amides. Due to the steric effects, tertiary amines do not yield stable derivatives when subjected to acid chlorides and therefore, are not included in this study.

The presence of the reaction product, after solvent extraction into $CHCl_3$, gave intensely absorbed signals by UV spectrophotometry. The derivatization procedure involves the drying of the derivatized product and hence preconcentrates the dilute sample. The mass spectra and the elemental analysis of the products confirmed the conversion of the amine to the sulfonamide. The peak which represents the M^+ or $(M+1)^+$ ion for all the sulfonamides formed were detected, with an intensity of less than 1%. The base peak also found at m/z 129 which represents the fragment of $C_9H_7N^+$ after the cleavage of C–S bond. The elemental analysis further indicates that the mass percentage of C, H and N atoms in the sample isolated was in accordance with the molecular mass of the corresponding sulfonamide, with the error less than 0.4%.

3.2. Relationship between retention and the incremental carbon number

In two different mobile phases tested, containing MeOH–acetate buffer–TEA and MeCN–acetate buffer–TEA in different compositions, one common trend was observed for both the RP-C_6 and RP-CN columns used, the retention generally decreases when the composition of the organic modifier increases. However, the retention of the 8-quinolinesulfonamides on cyano column was too close and thus separation was unsuccessful. This trend was observed for both MeOH and MeCN when they were used as the organic modifier. On the other hand, when the RP-C_6 column was used, although the difference between each retention factor is not too large, it is sufficient for most of the 8-quinolinesulfonamides to be separated. This is shown in Figs. 1 and 2. The optimum condition for the separation was achieved at the MeCN–acetate buffer–TEA ratio of 50:50:0.01. As shown in Figs. 1 and 2, the selectivity decreases with increases of the composition of organic modifier and was found poor

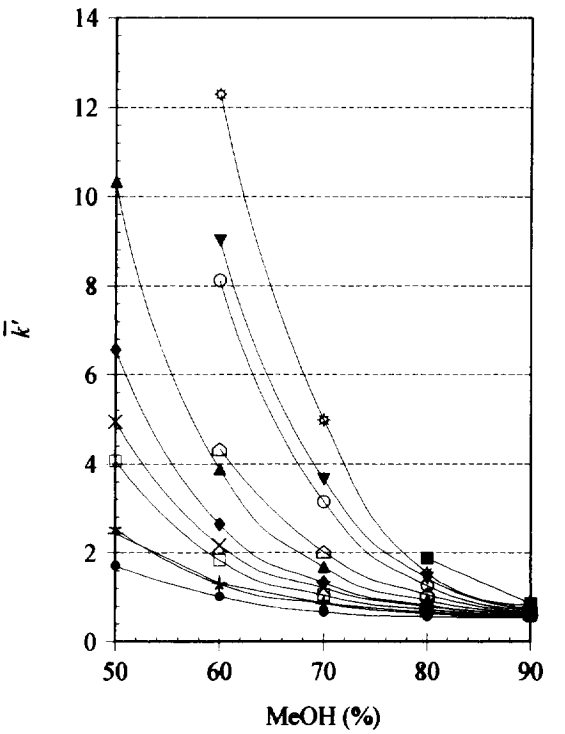


Fig. 1. Plots of the mean of capacity factor, $\bar{k}'$ vs. the composition of the organic modifier, MeOH (%) after the elution on an RP-C_6 column. Derivative of ● = methylamine; + = dimethylamine; * = ethylamine; □ = propylamine; × = diethylamine; ♦ = butylamine; ▲ = pentylamine; ⬠ = dipropylamine; ○ = heptylamine; ▼ = dibutylamine; ☆ = octylamine; ■ = nonylamine.

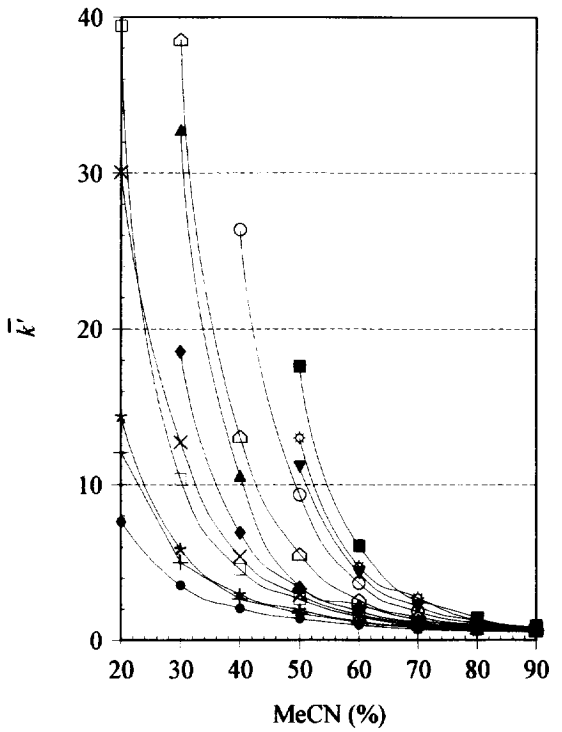


Fig. 2. Plots of the mean of capacity factor, $\bar{k}'$ vs. the composition of the organic modifier, MeCN (%) after the elution on an RP-C_6 column. Symbols used are the same as in Fig. 1.

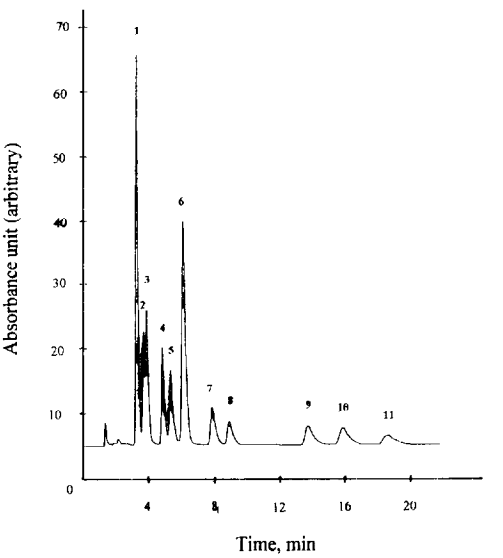


Fig. 3. Liquid chromatogram of 8-quinolinesulfonamides on a RP-C_6 column, with MeCN–acetate buffer–TEA (50:50:0.01). Sulfonamide of 1 = methylamine; 2 = dimethylamine; 3 = ethylamine; 4 = propylamine; 5 = diethylamine; 6 = butylamine; 7 = pentylamine; 8 = dipropylamine; 9 = heptylamine; 10 = dibutylamine; 11 = octylamine. (This result has been presented in Malaysian Science and Technology Congress 1994)

when the composition is greater than about 80% for MeOH and 60% for MeCN when they were used with the RP-C_6 column.

The optimum separation of the amine derivatives achieved with the MeCN–acetate buffer–TEA ratio of 50:50:0.01 is shown in Fig. 3. Separation was accomplished with almost all the separation factors, α better than 1.10 with separation time within 20 min. The separation achieved was satisfactory in terms of the α values since in most practical environments, values of 1.05 are sufficient for LC separation. The α values are listed in Table 1.

When MeOH was used as the organic modifier in the separation, selectivity observed was not as good as when MeCN was used as the organic modifier. One of the chromatograms showing the results when MeOH was used is given in Fig. 4.

Further investigation on the plots of log k' vs. the incremental carbon number, n_c strongly suggested a linear dependence between log k' and n_c with an excellent agreement ($r \geq 0.99$). Two examples of the plots are shown in Figs. 5 and 6, with a common intersection point clearly seen. Similar results were also observed by Grushka et al. [21] with alkylbenzenes as the solutes and RP-C_{18} as stationary phase. As discussed in that paper, the physical

Table 1
Separation factor, α obtained in the separation of 8-quinolinesulfonamides by using the RP-C_6 column, with MeCN–acetate buffer–TEA (50:50:0.01)

Peak of the 8-quinolinesulfonamides		α
First peak	Second peak	
Methylamine	Dimethylamine	1.21
Dimethylamine	Ethylamine	1.09
Ethylamine	Propylamine	1.38
Propylamine	Diethylamine	1.13
Diethylamine	Butylamine	1.19
Butylamine	Pentylamine	1.36
Pentylamine	Dipropylamine	1.16
Dipropylamine	Heptylamine	1.63
Heptylamine	Dibutylamine	1.17
Dibutylamine	Octylamine	1.19

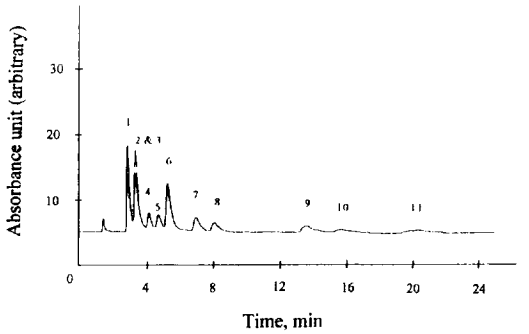


Fig. 4. Liquid chromatogram of 8-quinolinesulfonamides on a RP-C_6 column, with MeOH–acetate buffer–TEA (60:40:0.01). Sulfonamide of 1 = methylamine; 2 = dimethylamine; 3 = ethylamine; 4 = propylamine; 5 = diethylamine; 6 = butylamine; 7 = pentylamine; 8 = dipropylamine; 9 = heptylamine; 10 = dibutylamine; 11 = octylamine.

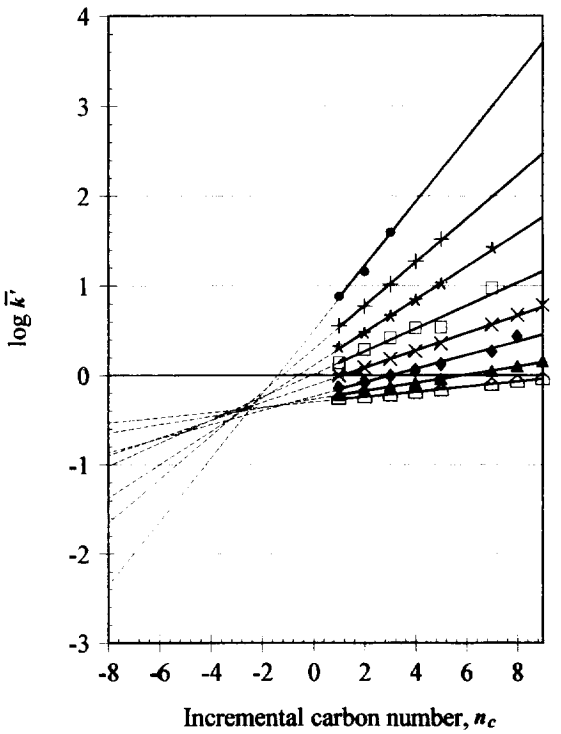


Fig. 5. log $\bar{k}'$ vs. the incremental carbon number, n_c plots showing a common intersection point for primary amine derivatives obtained on RP-C_6 column when MeCN is used as the organic modifier. MeCN–acetate buffer–TEA ratios are ● = 20:80:0.01; + = 30:70:0.01; * = 40:60:0.01; □ = 50:50:0.01; × = 60:40:0.01; ♦ = 70:30:0.01; ▲ = 80:20:0.01; ○ = 90:10:0.01 (v/v).

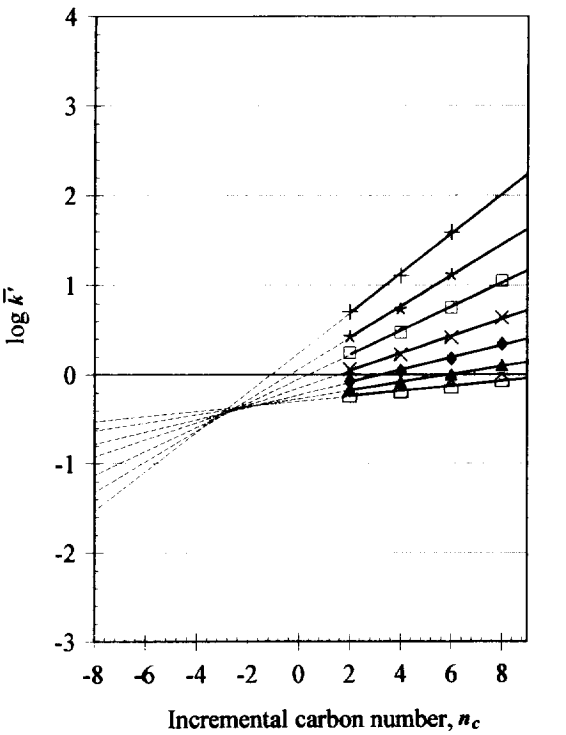


Fig. 6. log $\bar{k}'$ vs. the incremental carbon number, n_c plots showing a common intersection point for secondary amine derivatives obtained on RP-C_6 column when MeCN is used as the organic modifier. Symbols used are the same as in Fig. 5.

significance of that point is not yet clear, but its existence can be taken as an indicator of the linearity of the dependence observed. The equations obtained through linear regression analysis are listed in Tables 2 and 3.

4. Conclusions

The system studied was found to be good for the separation of eleven low-molecular-mass aliphatic primary and secondary amines, with satisfactory results achieved within 20 min. Since the linear relationships observed between the retention and the incremental carbon number of sulfonamides were excellent with $r \geq 0.99$, it is possible to predict the retention of the amine derivatives using the equation obtained.

Table 2
Linear equation of amine 8-quinolinesulfonamides at different fixed composition of organic modifier on RP-C_6 column

Composition (%) of MeCN	Prediction equation for primary amine derivatives	Correlation coefficient, r for primary amine derivatives	Prediction equation for secondary amine derivatives	Correlation coefficient, r for secondary amine derivatives
20	$0.357n_c+0.499$	0.991	–	–
30	$0.243n_c+0.295$	≥0.999	$0.222n_c-0.243$	0.999
40	$0.179n_c+0.127$	≥0.999	$0.174n_c-0.063$	0.998
50	$0.138n_c+0.002$	≥0.999	$0.135n_c-0.050$	0.998
60	$0.091n_c-0.094$	≥0.999	$0.096n_c-0.151$	0.998
70	$0.068n_c-0.211$	≥0.999	$0.069n_c-0.227$	0.997
80	$0.044n_c-0.265$	≥0.999	$0.045n_c-0.265$	0.997
90	$0.029n_c-0.302$	0.996	$0.028n_c-0.298$	0.996

Table 3
Linear equation of amine 8-quinolinesulfonamides at different fixed composition of organic modifier on RP-CN column

Composition (%) of MeCN	Prediction equation for primary amine derivatives	Correlation coefficient, r for primary amine derivatives	Prediction equation for secondary amine derivatives	Correlation coefficient, r for secondary amine derivatives
10	$0.110n_c-0.052$	0.989	$0.168n_c-0.160$	0.987
20	$0.062n_c-0.137$	0.993	$0.122n_c-0.317$	0.966
30	$0.049n_c-0.197$	0.987	$0.064n_c-0.265$	0.963
40	$0.038n_c-0.244$	0.981	$0.027n_c-0.216$	0.986
50	$0.011n_c-0.252$	0.996	$0.010n_c-0.246$	0.991

Acknowledgments

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