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Effect of mobile phase anionic additives on selectivity, efficiency, and sample loading capacity of cationic drugs in reversed-phase liquid chromatography

Jun Dai¹ and Peter W. Carr^{*}

Department of Chemistry, University of Minnesota, 207 Pleasant Street SE., Minneapolis, MN 55455, USA

Abstract

In the present work, we study the effect of mobile phase anionic additives type and concentration on selectivity, efficiency, and sample loading capacity of cationic drugs in reversed-phase liquid chromatography (RPLC).

The type and concentration of an anionic additive are known to have a strong effect on the absolute retention of cations in RPLC; in contrast they have only a small effect on the selectivity of one cation relative to a second as seen here. This is mainly due to the similarity of the ion pair formation constants between the selected cations. The limiting retention factors of cations (i.e. the retention factor of the fully ion-paired analyte at very high additive concentration) are roughly proportional to their inherent hydrophobicities (i.e. the retention factor of the analyte in the absence of the anionic additive). With a given anion, differences in ion pairing strength between the solutes are required for effective selectivity adjustment.

Based on the Wade-Lucy-Carr (W-L-C) kinetic model of overload peaks, the approach we developed in our previous work was used to study the effect of mobile phase anionic additives type and concentration on the limiting plate count (N_0) and sample loading capacity ($\omega_{0.5}$) of various cationic drugs. Under linear chromatographic conditions, where the analyte exhibits its smallest peak width and thus maximum apparent plate count, the type and concentration of anionic additives have almost no effect on peak width. In comparison to neutral analytes the sorption isotherms of cationic species are very easily overloaded even when many fewer moles of cations as compared to neutrals are injected.

We showed that different anionic additives profoundly affect the cations' "overload profiles" (i.e. plots of plate count versus amount injected) by changing the sample loading capacities. The increase in sample loading capacities with different anions shows the same order as the extent of ion pairing between the anions and the basic analytes. The detrimental effect of sample overloading on peak width can be greatly diminished by using either a stronger ion pairing agent or a higher concentration of a given ion pairing agent. Both effects operate by increasing the sample loading capacity, thereby allowing more solute to be injected. We believe that the increase in sample loading capacity described above is due in part to the increase in number of ion-exchange sites as more anions sorb to the stationary phase. At the same time, the formation of a

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^{*}To whom correspondence should be addressed (petecarr@umn.edu).

¹current address: Bristol-Myers Squibb, Route 206 and Province Line Road, Princeton, NJ 08540, USA

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neutral ion-paired analyte also increases the amount of cation which can be loaded onto the stationary phase by allowing a greater fraction of the analyte to be present in the stationary phase as an electrically neutral (i.e. ion-paired) species.

1. Introduction

Mobile phase anionic additives significantly affect the retention of cations in RPLC but have only a very slight influence on the retention of neutral compounds [1,2]. In addition to their effect on retention, it is also important to understand their frequently overlooked effect on selectivity, efficiency, and sample loading capacity of cations, which are very critical factors for the separation of cations in RPLC.

1.1 Effect of anionic additives on the selectivity of cations

Despite the well known profound effect of anionic additives on the retention of cations [3,4], there have been relatively few studies of their effect on selectivity. Theoretical guidance in selectivity adjustment would be very useful for choosing mobile phase additives to improve these separations.

In his review, Fritz divided the ion pairing ability of sample anions into two components: electrostatic attraction and “enforced pairing” [5]. Electrostatic attraction is more important for the small, hydrophilic, typically inorganic, anions with a high charge. According to the so called “enforced pairing” effect, stronger ion pairing occurs within the stationary phase due to a combination of effects that may include hydrophobic interaction, hydrogen bonding, a lower dielectric constant, and “water-structure induced ion pairing”. The enforced pairing effect was said to be the major reason for variations in selectivity due to changes in hydrophobicity/hydrophilicity.

Hodges and coworkers have studied the effect of ion pairing agent concentration on the RPLC elution behavior of peptides [6,7]. They have shown that ion pair agents (e.g. trifluoroacetic acid and pentafluoropropionic acid) are more effective in varying the elution order of peptides with different charges compared to those having the same charge. However, a detail interpretation has not been provided for these observations.

Using ion pair formation constants measured by capillary electrophoresis, we have clarified the role of ion pairing in the retention of cation separations in RPLC [1,2]. Our studies show that the anion can influence the retention of cations by either ion pair formation in the mobile phase or simultaneous contributions from both ion pair formation in the mobile phase and dynamic ion-exchange in the stationary phase.

We have shown that the retention of cations can be expressed by the following equations [1]:

1. Pure ion pair formation in the mobile phase

$$k' = \beta \frac{[A^+ : X^-]_s + [A^+]_s}{[A^+]_m + [A^+ : X^-]_m} = \beta \frac{K_{D,ip} K_{ip} [X^-]_m + K_{D,0}}{1 + K_{ip} [X^-]_m} = \frac{k'_{\max} K_{ip} [X^-]_m + k'_0}{1 + K_{ip} [X^-]_m} \quad (1)$$

where β is the phase ratio, K_{ip} is the ion pair formation constant between the analyte A^+ and anionic additive X^- , $K_{D,ip}$ is the distribution constant of the ion-paired analyte between the stationary and mobile phases, $K_{D,0}$ is the distribution constant of the analyte at zero concentration of X^- , $[X^-]_m$ is the concentration of anionic additive, k' is the solute retention factor in the presence of the additive, k'_0

is the retention factor in its absence, $k'_{\max}$ is the limiting retention factor of the fully ion-paired solute at very high additive concentration, and the subscripts m and s denote the mobile and stationary phases.

2. Pure dynamic ion-exchange

$$k' = \beta \frac{[A^+X^-]_s + [A^+]_s}{[A^+]_m} = \beta \frac{K_{\text{EX,AX}} K_{\text{D,CX}} [X^-]_m + K_{\text{D,0}}}{1 + K_{\text{D,CX}} [X^-]_m [C^+]_m} = \frac{k'_{\max} K_{\text{D,CX}} [X^-]_m + k'_0}{1 + K_{\text{D,CX}} [X^-]_m [C^+]_m} \quad (2)$$

where $K_{\text{D,CX}}$ is the distribution constant for the additive between the stationary and mobile phases, $K_{\text{EX,AX}}$ is the equilibrium constant for the ion-exchange process of the analyte in the mobile phase and the sorbed anionic additive in the stationary phase, C^+ is the counterion (e.g. Na^+) associated with the anionic additive, and $k'_{\max}$ is the limiting retention factor observed at very strong sorption of the additive to the stationary phase.

3. Simultaneous contribution from ion pair formation and dynamic ion-exchange

$$k' = \frac{K[X^-]_m + k'_0}{(1 + K_{\text{ip}}[X^-]_m)(1 + K_{\text{D,XC}}[X^-]_m[C^+]_m)} \quad (3)$$

where K is the product of distribution and equilibrium constants.

Based on inspection of Eq. 1, we see that ion pair formation in the mobile phase acts in two opposing fashions. The term $k'_{\max} K_{\text{ip}}[X^-]_m$ in the numerator clearly indicates that an increase in $K_{\text{ip}}[X^-]_m$ will increase retention. However, the term $(1 + K_{\text{ip}}[X^-]_m)$ appears in the denominator. This leads to the decrease in retention corresponding to the decrease in the availability of X^- in the mobile phase which can then no longer provide dynamic ion-exchange sites in the stationary phase. It is this second process that Knox and Hartwick in their seminal paper on the impact of mobile phase ion interaction agents said gave a decrease in solute retention [8].

However, the retention enhancement due to $k'_{\max} K_{\text{ip}}$ drives the significant increase in retention of cations when a small amount of the inorganic anions of interest here are added to the mobile phase.

If we consider the selectivity (α) of two cationic analytes A^+_1 and A^+_2 under pure mobile phase ion pair formation mechanism, the following equation is obtained

$$\alpha_{A_1/A_2} = \frac{(k'_{\max} K_{\text{ip}}/k'_0 + 1/[X^-]_m)_{A_1}}{(k'_{\max} K_{\text{ip}}/k'_0 + 1/[X^-]_m)_{A_2}} \times \frac{(1/[X^-]_m + K_{\text{ip}})_{A_2}}{(1/[X^-]_m + K_{\text{ip}})_{A_1}} \alpha_0 \quad (4)$$

where α_0 is the selectivity in the absence of the additive.

Based on our previous studies, under certain conditions, the measured ion pair formation constants of different cations with a given anion were very similar [1,2]. If we assume they are exactly equal, eq. 4 reduces to:

$$\alpha_{A_1/A_2} \approx \frac{(k'_{\max} K_{ip}/k'_0 + 1/[X^-]_m)_{A_1}}{(k'_{\max} K_{ip}/k'_0 + 1/[X^-]_m)_{A_2}} \alpha_0 \quad (5)$$

We see that it is principally the ratio of $k'_{\max} K_{ip}/k'_0$ that determines the relative selectivity of two cations in the presence of an ion pair agent. Eqs. 4 and 5 are also applicable to the dual retention mechanism (Eq. 3) since the sorption of the anionic additive to the stationary phase is independent of the analyte.

1.2 Effect of anionic additives on the efficiency and sample loading capacity of cations

Peak shape and width are very important in RPLC for resolution enhancement and separation improvement [9,10]. Many factors contribute to the peak shape and width; they include: eddy dispersion, slow kinetics of desorption, and the overloading of the sorption isotherm (i.e. non-linear behavior in the equilibrium relationship between the analyte concentration in the stationary and mobile phases [11–15].

The general consensus (but see below for more detail) concerning peak tailing of cationic bases in RPLC is that even at low pH it is due to strong electrostatic interactions between a very small population of ionized silanol sites and positively charged analytes [16,17]. Wirth and coworkers have also suggested that the interaction is not electrostatic but involve neutral isolated silanol sites [18,19]. Non-electrostatic interactions have been blamed for peak tailing for some uncharged polar compounds [20,21].

The detrimental effect of excessive sample load is often ignored as a source of peak broadening under typical analytical conditions although this effect has been explored very extensively by Guiochon [14,15,22–26] and McCalley [27–29]. Under linear isotherm conditions, the amount of sample applied has no effect on the retention and peak width as long as an excessive volume is not used and the injection solvent is well matched to the eluent. When a large mass of sample is injected, peak distortion will be observed [9]. The existence of a high sample loading range for linear chromatographic conditions is very important for efficient and robust chromatographic separations. However, the linear range is generally much smaller for cations compared to neutral compounds [12,30,31]

The overloading of high energy-low population adsorption sites strongly affects the peak width. Gritti and Guiochon have studied the surface heterogeneity and concluded that for very polar but neutral species (caffeine) there are several different adsorption sites and a very low population of very high energy sites is responsible for the very small linear chromatographic range [26]. Based on the very different saturation capacities for caffeine obtained on different stationary phases, they also concluded that the loading capacity strongly depends on the experimental conditions.

McCalley has shown that higher mobile phase pHs can provide higher sample load for cations compared to low pH conditions due to the different number of ionized silanol sites on the reversed-phase stationary phase [27,32]. He concluded that silanol interactions alone do not fully explain the overload behavior of bases since ionized silanol groups are minimal at low pH conditions [29,33] (e.g. for one of the phases he used it has been shown that there are virtually no ionized silanol sites below pH 7 [34]). To explain tailing he invokes a mutual repulsion effect between sorbed cations as proposed previously by Snyder and coworkers [12,30] as the major process causing the overloading of bases at low pH [29,35]. His conclusion is supported by the fact that negatively charged analytes also tend to be

easily overloaded and show tailed peak at very low sample loads despite the fact that they do not interact with silanol groups [29].

The presence of mobile phase anionic additives strongly affects the sample overload behavior. It has been shown that use of dilute (0.05–0.1%) formic acid eluents gives much lower apparent plate counts for cations compared to other conventional buffers such as phosphoric acid buffers (20–50 mM) [28,29]. Lower sample loading capacity due to low ionic strength and very weak ion pairing effects in formate media were said to be responsible for the poor peak shapes and low plate counts.

Similarly, Gritti and Guiochon found that the addition of salts and increases in salt concentration alter the isotherm and improve the sample saturation capacity. Ion pair formation between the charged analytes and the mobile phase additives was invoked to rationalize the phenomena [36]. Based on experimental results obtained on different bonded phases, they also concluded that the loading capacity of ionized analytes increases with increasing salt concentration in the mobile phase due to a decrease in the repulsive interaction among the sorbed analytes. In addition, they used specific salt-solute electrostatic interactions to explain the differences in adsorption behavior of ionizable analytes [25,37,38].

Pan et al. found that basic analytes show different apparent plate counts with various anionic additives. They also showed that higher concentrations of anionic additives improved the apparent plate counts [39].

Despite the numerous studies of mobile phase additive effects on the peak width and sample load of ionizable analyte, quantitative studies were seldom performed to compare the effect of various additive types and concentrations. One of the main purposes of the present work is to clarify the effect of anion type and concentration on plate count and loading capacity of cations by means of quantitative overload studies based on a simple but mathematically non-approximate Langmuirian kinetic equation (W-L-C) of peak overload [31,40–42].

2. Experimental

2.1 Instruments

All chromatographic experiments were carried out with a Hewlett-Packard 1090 equipped with a binary pump, a helium sparger, an autosampler, a thermostatted-column compartment, and a diode array detector (Agilent Technologies, Wilmington, DE, USA). Data were collected and processed using Agilent Chemstation software.

2.2 Analytical Columns

The stable bond (SB) C₁₈ column with dimensions of 50 mm × 4.6 mm I.D. was donated by Agilent Technologies (Wilmington, DE, USA). The average particle size was 5 µm and the average pore diameter was 80 Å.

2.3 Reagents

All chemicals were reagent grade or better. Basic drugs (see Fig. 1 for structures) were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile was from Burdick & Jackson (Muskegon, MI, USA). HPLC water was obtained from a Barnsted Nanopure deionizing system (Dubuque, IA, USA) and run through an “organic-free” cartridge followed by a 0.2 µm particle filter. The solution was then degassed under helium. All solvents were filtered through a 0.2 µm filter (Lida Manufacturing, Kenosha, WI, USA).

before use. Other chemicals used in this study were purchased from Aldrich Milwaukee, WI, USA).

2.4 Chromatographic Conditions

All chromatographic measurements were made at a flow rate of 1 mL / min., and detection was set at 254 nm. The column temperature was controlled to 35.0 °C with the Hewlett-Packard 1090 oven. The dead time was determined by injecting uracil. The buffers at pH 4.8 were prepared from acetic acid and sodium acetate. 0.05–0.1% (v/v) formic acid was used for low pH conditions. We used acetate and formate buffers because compared to even chloride both are very weak ion pairing agents and weak displacing species in anion chromatography and thus they do not sorb onto the stationary phase [3,43,44]. Mobile phases with different additives were prepared by adding the sodium salts of each anion to the buffers. Sodium chloride was used to adjust the ionic strength for some of the experiments. The concentrations of buffer and sodium salts were reported with respect to the volume of the aqueous-organic mixture. The cation samples with different concentrations are prepared in 35/65 acetonitrile/water. The injection volume was 1 µL.

3. Results and discussion

3.1 Effect of anionic additive type and concentration on the selectivity of cationic drugs

Fig. 2 shows the effect of anion type on the selectivity of cations at pH 4.8 and 2.8. At pH 4.8, retention factors of cations with different anionic additives show strong correlations in the “κ-κ” plots [45]. At pH 2.8 there is more scatter, specifically for perphenazine (see Fig. 2B). The selectivity evaluated in terms of κ-κ plots of a large group of cations at pH 2.8 was compared in Fig. 3. Although retention factors vary significantly, the differences in selectivity are not dramatic.

Fig. 4 shows the selectivity of different analytes versus amitriptyline under various mobile phase conditions. As can be seen, except for perphenazine at pH 2.8, the retention factors of all cations are strongly correlated at both pH 4.8 and 2.8. More scatter (i.e. greater changes in selectivity) exist for perphenazine at pH 2.8 (see Fig. 4B).

From Figs. 2 to 4, it seems that variations in the type and concentration of the anion additives do not really change the selectivity among different cations. Given the significant effect of anion type and concentration on the absolute retention of these cations, we did not anticipate the minor concomitant effect of anion on selectivity.

As discussed in the introduction, the ratio of $k'_{\max}K_{ip}/k'_0$ and differences in ion pair formation constants are the critical factors that influence the selectivity. Table 1 compares the effect of anionic additive type on the K_{ip} and $k'_{\max}$ values obtained at pH 4.8 [1]. For a given anion the changes in the ion pair formation constants as the cation is varied are much smaller than the changes in the ion pair formation constants observed as the anion is varied with a given cation. Thus to a first approximation the variations in the strength of ion pairing are governed by the anion and not by the cation. More importantly, the ratio $k'_{\max}/k'_0$ is almost independent of the particular cation, indicating that $k'_{\max}$ of the ion-paired cation is related to the hydrophobicity (i.e. k'_0) of the cation. Under these conditions, adjusting the relative selectivity of two cations by the use of an anionic additive is difficult. Eq. 5 can be rewritten as:

$$\alpha_{A_1/A_2} \approx \frac{(k'_{\max}K_{ip}/k'_0 + 1/[X^-]_m)_{A_1}}{(k'_{\max}K_{ip}/k'_0 + 1/[X^-]_m)_{A_2}} \alpha_0 \approx \alpha_0 \quad (6)$$

Although different anions have very different ion pairing ability, their net effect on relative retention of different cations seems to be rather small. From Table 1, it becomes clear that the limitation in adjusting selectivity under various conditions as seen in Figs. 2 to 4 is due to the similarity of ion pair formation constants among the cations and the similarity in the ratio of $k'_{\max}/k'_0$. We are not sure of the cause of the similarity of $k'_{\max}/k'_0$ for all the cations and have no firm explanation. All we can say for certain is the data show that whatever chemical characteristic of the cation controls its inherent, that is, unassisted by the anionic mobile phase additive, tendency to sorb to the stationary phase from the eluent also participates in the cation's tendency to ion pair in the mobile phase and then sorb to the stationary phase. The two chief characteristics of the organic cation that relate to their retention are their hydrophobicities and Coulombic interactions. To a first approximation the charge and the size of the positively charged group on the drugs are very similar. This leads us to hypothesize that the differences in the hydrophobicities (or lipophilicities) of the various organic cations contribute similarly to k'_0 and $k'_{\max}$.

When the types of analytes are different, e.g. diamines versus monoamines or neutral versus basic compounds, the analytes will pair to rather different extents. As seen in Fig. 4B, relatively large changes in selectivity were obtained for perphenazine at pH 2.8. Since perphenazine (pK_a 3.7 and 7.8) is the only diamine studied and a considerable fraction must be doubly charged at pH 2.8, we believe that the selectivity change is mainly due to the change in its ion pairing ability as compared to the singly charged amitriptyline.

Fig. 5 shows the effect of anion concentration on the selectivity between a neutral analyte (benzene), a monoamine (amitriptyline pK_a 9.4), and a diamine (perphenazine). As perphenazine becomes a dication at pH 2.8, the different ion pairing strength allows its selectivity relative to the monoamine to be tuned over a significant range; this cannot be achieved at pH 4.8 as both analytes are monoamines at the higher pH. Fig. 5 clearly demonstrates that effective selectivity adjustment between different types of analytes can be achieved. Our results are consistent with those of Shibue et al. on peptides [7] in that the ion pairing agent has a greater impact on selectivity for analytes with different charge states.

3.2 Effect of anionic additive type and concentration on the overload profile of cationic drugs

In a seminal study of peak shape in linear chromatography of tailed peaks, Lenhoff showed that the use of peak moments and simple parameters such as peak width alone could be very problematic and deceptive [13]. Nonetheless in this work we opted to make the simpler measurements and assumed that comparisons would be valid. Based on our recent theoretical studies of sample overloading effects [42] and those of others [46,47], there are two key related parameters that must be defined to characterize the overload behavior of an analyte: the limiting plate count and the column sample loading capacity.

To quantitatively compare the overload behavior of different analytes under different conditions, we define the sample load (ω) at which the apparent plate count (N) drops to half of its limiting value (i.e. N_0 the maximum plate count which is obtained under linear chromatographic conditions) as the analytical operational sample loading capacity (i.e. $\omega_{0.5}$) [42]. To obtain reliable N_0 values and overcome the low signal/noise ratio problem, we developed a model equation (see Eq. 7) to fit experimental data obtained at higher concentrations where the signal/noise ratio is better to get the two key parameters by use of a non-linear fitting procedure [42]. The variation in N as a function of ω was determined by using the equation governing the shape of the concentration time curve based on the Wade-Lucy-Carr (W-L-C) kinetic model of peak overload [31,40–42]. An example of overload peak profiles based on the dimensionless overload parameter θ (note θ is defined by the W-

L-C model) [42] is shown in Fig. 6A. A “universal overloading curve” was obtained for the relative plate count (N/N_0) as a function of relative sample load ($\omega/\omega_{0.5}$):

$$\frac{N}{N_0} = \frac{1 + 1.49\omega'}{1 + 1.49\omega' + 2.49\omega'^2} \quad (7)$$

where $\omega' = \omega/\omega_{0.5}$ is the relative sample load.

As shown in Figs. 7 and 8, the theoretically predicted plate counts (the solid curves) based on Eq. 7 agree very well with the experimental data (symbols). After normalization, the overload behavior of the various analytes under all conditions follows essentially the same curve, i.e. Eq. 7 (see Fig. 9). A more detailed description of the method used to obtain the sample loading capacity and limiting plate counts is presented in our previous work [42].

As seen from Fig. 7, the type of anionic additive has a significant effect on a probe cation's overload “profile” (i.e. plot of N vs. amount injected). At the same anion concentration, a clear trend in sample load, independent of cation, is observed for different types of anions: $\text{Cl}^- < \text{CF}_3\text{COO}^- < \text{ClO}_4^- < \text{PF}_6^-$. Similarly, at constant ionic strength, for the same type of anion (i.e. perchlorate), the sample loading capacity increases with concentration of the ion pairing anion (see Fig. 8): $0\text{mM} < 40\text{mM} < 60\text{mM}$. Within the concentration range used here, the higher is the concentration of anion, the narrower is the peak width at the same sample load.

The pattern of sample overloading behavior is consistent with the extent of ion pairing of the anion and the cations [2]. The greater is the degree of ion pairing (either by the use of a stronger ion pair anion or a higher anion concentration), the higher is the apparent plate count of the peak at the same sample load.

Fig. 10 shows the effect of anion type and sample load on the retention of cations. Although the anion type has a significant effect on the retention of cations, the effect of sample load on retention is rather small.

3.3 Effect of anionic additive type and concentration on limiting plate count and the sample loading capacity of cationic drugs

Tables 2 and 3 show the effect of anion type and concentration on the limiting plate count, sample loading capacity, and retention, respectively. Clearly, anion type and concentration have a significant impact on both retention and sample loading capacity. The trends are consistent with the extent of ion pairing with the type and concentration of the various anions. In contrast to their significant influence on retention and sample loading capacity of cations, mobile phase conditions have a much smaller effect on the limiting plate count (N_0) where the sample loading behavior is still linear, especially when the cation is relatively well retained.

A dramatic difference in sample loading capacities is observed when formate buffers were used (see Table 4). The added ion pairing salt (i.e. sodium trifluoroacetate) significantly increased the sample loading capacity. Also the limiting plate counts are relatively high in the presence of an ion pairing salt. The effect of mobile phase pH on sample loading capacity and limiting plate counts are compared in Table 5. Lower values of N_0 and higher sample loading capacities are obtained at the higher pH.

Based on the results in Tables 2 to 5, we see that mobile phase conditions significantly affect the sample loading capacity but they only have a small effect on the limiting plate count despite big differences in retention factors. The stronger ion pairing agents increase the

sample loading capacity in parallel with their ability to increase retention. A higher loading capacity is achieved by using either a stronger ion pairing anion (Table 2) or a higher anion concentration (Table 3).

It seems that the addition of an ion pairing agent increases the sample loading capacity by increasing the retention of the cations. As seen in Fig. 11, loading capacity increases almost linearly with retention. However, Fig. 11 also shows that the increase in sample loading capacity as a function of k' is relatively greater for the less retained analytes, e.g. the slope of k' vs. $\omega_{0.5}$ is greater for alprenolol than that for amitriptyline. Most interestingly, 60 mM sodium chloride provides a higher sample loading capacity than does 20 mM sodium chloride despite, as we previously noted and explained [1], the fact that an increase in sodium chloride concentration actually decreases retention (see Table 6 and comments below).

Previously we had shown in our theoretical studies, which are in good agreement with those of Snyder et al. [47] and Knox/Pyper [46], that greater retention does not *per se* mean an increase in the sample loading capacity [42]. In fact for k' values greater than 2–3 there is very little increase in $\omega_{0.5}$ as k' is increased further based on W-L-C model [42].

This is in sharp contradistinction to our experimental observations in Fig. 11. It is clear that other chemical factors which correlate with k' contribute to the increase in sample loading capacity and these factors are not present in the purely mathematical W-L-C model. We believe these factors are the result of two processes.

First, as the concentration of ion pairing anion in the eluent is increased or a stronger (less well hydrated) ion pair agent is used, the amount of anion that sorbs into the stationary phase increases [1]. The correlation between ease of ion pair formation and ease of anion sorption has been responsible for considerable confusion in the past. Based on our previous studies, we know that those anions which form stronger ion pairs also sorb much more easily into the stationary phase [1]. When an anion which sorbs more readily to the surface (and coincidentally forms stronger ion pairs with the analyte) or a higher concentration of that anion is used, the increase in amount of anion sorption creates more interaction sites for the analyte cation in the stationary phase. Consequently both the analyte's retention factor and the number of sorption site hence its loading capacity increase.

Second, even ignoring sorption of the anion and the concomitant formation of dynamic ion exchange sites, a greater fraction of the analyte in the stationary phase will be present as ion pairs when one uses either a high concentration of the anion or a stronger ion pair anion. The fraction of the analyte in the stationary phase that is ion paired can be derived as follows:

$$\alpha_{\text{ion pair},s} = \frac{[A^+:X^-]_s}{[A^+]_s + [A^+:X^-]_s} \quad (8)$$

According to Eq. 1, Eq. 8 can be rewritten as

$$\alpha_{\text{ion pair},s} = \frac{\beta[A^+:X^-]_s/[A^+]_m}{k'_0 + \beta[A^+:X^-]_s/[A^+]_m} = \frac{k'_{\text{max}}K_{ip}[X^-]_m}{k'_0 + k'_{\text{max}}K_{ip}[X^-]_m} \quad (9)$$

From Eq. 9, we can see that both strong ion pairing anion (large K_{ip}) and higher anion concentration lead to a greater fraction of ion-paired analyte in the stationary phase. A comparison of ion pairing for different anions is shown in Table 7. Although the extent of

ion pairing in the mobile phase is surprisingly small [1], ion pairing in the stationary phase is very strong.

The unusual behavior in chloride media in Table 6 indicates that anion sorption and ion pair formation are not the only mechanisms for increasing the loading capacity. Chloride is a weak ion pairing agent and does not sorb at all well to the stationary phase, yet an increase in the concentration of sodium chloride significantly improved the sample loading capacity but simultaneously decreased the sample k' . We believe that the higher ionic strength modifies the sample loading capacity by decreasing the strength of electrostatic interactions between analyte cations in the Gouy–Chapman double layer by “screening” the mutual cationic charges in accord with basic theory [48,49]. Thus, higher ionic strength also improves the sample loading capacity. This is fully consistent with McCalley’s “repulsion model” of overload of charged species [29].

However, ionic strength and purely electrostatic interactions alone cannot explain all the results many of which are obviously chemically specific to the individual anions. The loading capacity does vary with different types and concentration of additives even when ionic strength is held constant (see Tables 2 and 3). Table 6 also shows that in the same ionic strength range, the change in sample loading capacity is not the same for a weak ion pairing agent (e.g. chloride) and a strong ion pairing agent (e.g. perchlorate).

Taking into account the differential sorption of anions onto the stationary phase, the formation of neutral ion-paired analytes, and ionic strength effects as discussed, we are not surprised to find the dramatic differences in the sample loading capacities with and without the ion pairing agent in a low ionic strength buffer (i.e. 0.05–0.1% formic acid) as seen in Table 4. Nevertheless, the overload process is very complex. It is conceivable that the overload profiles from the Langmuirian based W-L-C model (Fig. 6A) are different from the experimental results (Fig. 6B) in that the tailing parts in experimental data are not completely overlapped, possibly indicating non-Langmuirian behavior or the presence of multiple interaction sites as suggested by Guiochon [26]. Fig. 9 also shows that the fitting of Eq. 7 at very high overload is not as good as the fitting at moderate overload conditions.

Based on our previous study, we know that at pH 4.8 there are still a significant number of ionized silanol groups on the SB C₁₈ phase and that its silanophilicity becomes smaller at lower pH. [1] As seen in Table 5, higher silanophilicity gives higher sample loading capacity with slightly lower N_0 values. This is consistent with the literature [27]. With more ionized silanol groups, there are more interaction sites for basic analytes.

As seen in Tables 2 and 3, the limiting plate count of the analyte is almost independent on the anionic additive type and concentration provided that the analyte is well retained. This clearly shows that *under most conditions, the difference in sample loading capacity and sample overloading are the major reasons for different apparent plate counts* for cations.

However, under certain conditions, slow kinetics of adsorption also contributes to peak broadening. Conditions that are favorable for high energy interactions (i.e. silanophilic interactions) can lead to lower N_0 (e.g. the cases for pH 4.8 in Table 5).

4. CONCLUSIONS

We have compared the effect of mobile phase anionic additives on the selectivity, efficiency, and sample loading characteristics of cations in RPLC. Our studies provide useful guidance for both adjusting the selectivity and minimizing peak distortion through choice of the appropriate eluent conditions.

Based on our study, we conclude:

1. Type and concentration of anions only have a small effect on the selectivity of the cations studied. The limitation of selectivity adjustment for the selected cations is due to the similarity in their ion pair formation constants and in the ratio of $k'_{\max}/k'_0$ for the different cations studied. The change in selectivity when different type or higher concentrations of anions are used is greater for analytes with different charge states (e.g. diamines versus monoamines, basic versus neutral compounds).
2. The effects of type and concentration of anions on overload capacity of cations correlate with the anions' effect on the ion pair formation constant. Thus use of stronger ion pairing agents and higher anion concentrations give much greater sample loading capacities. We believe that anionic additives increase the sample loading capacity by effectively increasing the number of sites where the analyte cation can comfortably exist in the stationary phase. To a first approximation the stationary phase becomes overloaded by analyte cations due to mutual repulsion between charged sorbed species. The additive anions diminish this repulsion by reacting with the cations to form uncharged ion pairs with the cations or simply by adsorbing to the stationary phase and imparting a small but very important negative charge which serves to act as a sorption site for the cation.
3. Ion pairing in stationary phase is much stronger than in the mobile phase. Buffers which give very poor chromatographic performance (e.g. formate and acetate) are very weak ion pairing agents and also sorb very weakly to the stationary phase. Conversely anions which give very good sample loading characteristics (e.g. trifluoroacetate and perchlorate) are strong ion pairing agents and sorb strongly to the stationary phase.
4. The higher ionic strength increases the sample loading capacity.
5. Because sample loading capacity strongly depends on experimental conditions, great caution is needed when only plate counts is used for comparing the performance of different columns, analytes, or conditions. We believe that the sample loading capacity is fundamentally more important than the plate count.
6. The limiting plate counts which describe the peak profile under linear chromatographic conditions tend to be independent to mobile phase conditions provided that the analyte is reasonably retained.

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References

1. Dai J, Carr PW. J. of Chromatogr. A. 2005; 1072:169. [PubMed: 15887486]
2. Dai J, Mendonsa SD, Bowser MT, Lucy CA, Carr PW. J. of Chromatogr. A. 2005; 1069:225. [PubMed: 15830949]
3. Roberts JM, Diaz AR, Fortin DT, Friedle JM, Piper SD. Anal. Chem. 2002; 74:4927. [PubMed: 12380814]
4. Jones A, LoBrutto R, Kazakevich Y. J. of Chromatogr. A. 2002; 964:179. [PubMed: 12198846]
5. Fritz JS. J. of Chromatogr. A. 2005; 1085:8. [PubMed: 16106841]
6. Chen Y, Mant CT, Hodges RS. J. of Chromatogr. A. 2004; 1043:99. [PubMed: 15317418]
7. Shibue M, Mant CT, Hodges RS. J. of Chromatogr. A. 2005; 1080:58. [PubMed: 16013615]

8. Knox JH, Hartwick RA. *J. of Chromatogr. A.* 1981; 204:3.
9. Snyder, LR.; Glajch, JL.; Kirkland, JJ. *Practical HPLC Method Development*. New York: Wiley-Interscience; 1996.
10. McCalley DV. *J. of Chromatogr. A.* 1997; 769:169.
11. Giddings, JC. *Dynamics of Chromatography*. New York: Marcel Dekker Inc.; 1965.
12. Eble JE, Grob RL, Antle PE, Snyder LR. *J. of Chromatogr. A.* 1987; 384:45.
13. Lenhoff AM. *J. of Chromatogr. A.* 1987; 384:285.
14. Fornstedt T, Zhong G, Guiochon G. *J. of Chromatogr. A.* 1996; 741:1.
15. Fornstedt T, Zhong G, Guiochon G. *J. of Chromatogr. A.* 1996; 742:55.
16. Nawrocki J. *J. of Chromatogr. A.* 1997; 779:29.
17. Cox GB. *J. of Chromatogr. A.* 1993; 656:353.
18. Wirth MJ, Swinton DJ, Ludes MD. *J. of Phys. Chem. B.* 2003; 107:6258.
19. Smith EA, Wirth MJ. *J. of Chromatogr. A.* 2004; 1060:127. [PubMed: 15628155]
20. Verzele M, Dewaele C. *Chromatographia.* 1984; 18:84.
21. Engelhardt H, Arangio M, Lobert T. *LCGC.* 1997; 15:856.
22. Quinones I, Cavazzini A, Guiochon G. *J. of Chromatogr. A.* 2000; 877:1. [PubMed: 10845785]
23. Gritti F, Guiochon G. *J. of Chromatogr. A.* 2004; 1028:75. [PubMed: 14969283]
24. Gritti F, Guiochon G. *J. of Chromatogr. A.* 2004; 1028:197. [PubMed: 14989473]
25. Gritti F, Guiochon G. *J. of Chromatogr. A.* 2004; 1033:57. [PubMed: 15072290]
26. Gritti F, Guiochon G. *Anal. Chem.* 2005; 77:1020. [PubMed: 15858981]
27. McCalley DV. *J. of Chromatogr. A.* 1998; 793:31.
28. McCalley DV. *J. of Chromatogr. A.* 2004; 1038:77. [PubMed: 15233523]
29. McCalley DV. *Anal. Chem.* 2003; 75:3404. [PubMed: 14570190]
30. Cox GB, Snyder LR. *J. of Chromatogr. A.* 1989; 483:95.
31. Lucy CA, Wade JL, Carr PW. *J. of Chromatogr. A.* 1989; 484:61.
32. McCalley DV. *J. of Chromatogr. A.* 2000; 902:311. [PubMed: 11192164]
33. McCalley DV. *Anal. Chem.* 2006; 78:7. [PubMed: 16419324]
34. Mendez A, Bosch E, Roses M, Neue UD. *J. of Chromatogr. A.* 2003; 986:33. [PubMed: 12585320]
35. Buckenmaier SMC, McCalley DV, Euerby MR. *Anal. Chem.* 2002; 74:4672. [PubMed: 12349969]
36. Gritti F, Guiochon G. *J. of Chromatogr. A.* 2004; 1048:1. [PubMed: 15453413]
37. Gritti F, Guiochon G. *J. of Chromatogr. A.* 2004; 1033:43. [PubMed: 15072289]
38. Gritti F, Guiochon G. *J. of Chromatogr. A.* 2004; 1047:33. [PubMed: 15481458]
39. Pan L, LoBrutto R, Kazakevich YV, Thompson R. *J. of Chromatogr. A.* 2004; 1049:63. [PubMed: 15499918]
40. Wade JL, Bergold AF, Carr PW. *Anal. Chem.* 1987; 59:1286.
41. Lucy CA, Carr PW. *J. of Chromatogr. A.* 1991; 556:159.
42. Dai J, Carr PW, McCalley DV. *J. of Chromatogr. A.* 2009; 1216
43. Jacob LR. *Journal of Chromatography Library* 61(Protein Liquid Chromatography). 2000:235.
44. Karger, BL.; Snyder, LR.; Horvath, C. *An Introduction to Separation Science*. New York: Wiley-Interscience; 1973.
45. Melander W, Stoveken J, Horváth C. *J. of Chromatogr. A.* 1980; 199:35.
46. Knox JH, Pyper HM. *J. of Chromatogr. A.* 1986; 363:1.
47. Snyder LR, Cox GB, Antle PE. *Chromatographia.* 1987; 24:82.
48. Ståhlberg J. *J. of Chromatogr. A.* 1999; 855:3. [PubMed: 10514972]
49. Chen J-G, Weber SG, Glavina LL, Cantwell FF. *J. of Chromatogr. A.* 1993; 656:549.

Fig. 1.
Structures and pK_a s of the basic drugs.

Fig. 2.

Effect of anion type on the selectivity of basic drugs ("κ-κ" plots). Solutes, alprenolol, doxepin, desipramine, nortriptyline, amitriptyline, and perphenazine. Solutes are in the order of increasing retention factor under the same mobile phase condition except for perphenazine at pH 2.8 which was labeled with a "p" in the figure. Conditions: 35/65 acetonitrile/water with (A) 20 mM sodium acetate buffer at pH 4.8 and 20 mM NaX, (B) 0.05% formic acid at pH 2.8 and 20 mM NaX. Horizontal axis, logarithm of retention factor when $\text{X}=\text{Cl}^-$; vertical axis, logarithm of retention factor where $\text{X}=\text{PF}_6^-$ (Δ), $\text{X}=\text{ClO}_4^-$ (□), $\text{X}=\text{CF}_3\text{COO}^-$ (○), $\text{X}=\text{buffer only}$ (×).

Fig. 3.

Effect of anion type on the selectivity of basic drugs (“ κ - κ ” plot). Solutes, alprenolol, doxepin, desipramine, nortriptyline, amitriptyline, perphenazine, propranolol, oxprenolol, labetalol, metoprolol, acebueolol, and pindolol. Conditions: 35/65 acetonitrile/water, 0.05% formic acid at pH 2.8 with and without 20 mM NaPF₆.

Fig. 4.

Effect of anion type and concentration on the selectivity of basic drugs. Conditions: 35/65 acetonitrile/water, 20 mM sodium acetate at pH 4.8 with different concentration of additives (0–60 mM NaX with and without ionic strength adjustment from NaCl, X=Cl⁻, CF₃COO⁻, ClO₄⁻, PF₆⁻) or 0.05% formic acid at pH 2.8 with different concentration of additives (0–60 mM NaX with and without ionic strength adjustment from NaCl, X=Cl⁻, PF₆⁻; or 20 mM NaX with X= CF₃COO⁻, ClO₄⁻). Solute, (+) alprenolol at both pHs, ($\diamond$) doxepin at both pHs, ($\square$) desipramine at both pHs, ($\circ$) nortriptyline at both pHs, (Δ) perphenazine at pH 4.8, and ($\blacktriangle$) perphenazine at pH 2.8.

Fig. 5.

Effect of anion concentration on retention factor ratio of benzene and perphenazine relative to amitriptyline. Conditions: 35/65 acetonitrile/water, 0–60 mM NaPF_6 (constant ionic strength maintained by NaCl) with either 20 mM sodium acetate at pH 4.8 or 0.05% formic acid at pH 2.8. Plot symbols, (Δ) perphenazine at pH 4.8, ($\blacktriangle$) perphenazine at pH 2.8, ($*$) benzene at pH 4.8.

Fig. 6. Comparison of the effect of sample load on peak shape on theoretical and experimental chromatograms. (A) Peak profiles based on the W-L-C model ($k'=6$; $\gamma=1000$; $N_0=4080$; $\theta=1, 5, 10, 15$ and 20 . see ref. 42); (B) peak profiles based on experimental results of amitriptyline (35/65 acetonitrile/water, 0.05% formic acid, 0.05, 0.1, 0.2, 0.5, 1, and 2 nmol of sample, 254nm).

Fig. 7. Effect of anion type and sample amount injected on plate count of amitriptyline. Conditions: 35/65 acetonitrile/water, 20 mM sodium acetate at pH 4.8 and 20 mM NaX ($X = \text{Cl}^-$, CF_3COO^- , ClO_4^- , PF_6^-). Plot symbols, (Δ) PF_6^- , ($\square$) ClO_4^- , ($\circ$) CF_3COO^- , ($*$) Cl^- . Lines, predicted data by W-L-C model.

Fig. 8.
Effect of anion concentration and sample amount injected on plate count of amitriptyline.
Conditions: 35/65 acetonitrile/water, 20 mM sodium acetate at pH 4.8 and 0–60 mM
NaClO₄ (constant ionic strength maintained by NaCl). Plot symbols, NaClO₄ at (■) 60 mM,
(▲) 40 mM, (●) 0 mM. Lines, predicted data by W-L-C model.

Fig. 9.

Effect of normalized sample amount injected on normalized plate count. Solid line, theoretical dependence based on W-L-C kinetic model (i.e. Eq. 7); experimental data (+) alprenolol, (x) doxepin, (□) desipramine, (○) nortriptyline, (Δ) amitriptyline, and (◇) perphenazine. Conditions: 35/65 acetonitrile/water; 20 mM sodium acetate with either 20 mM NaX (X = Cl⁻, CF₃COO⁻, ClO₄⁻, PF₆⁻) or 0–60 mM NaClO₄ (constant salt concentration of 60 mM maintained by NaCl). N₀ and ω_{0.5} are based on Tables 2 and 3.

Fig. 10.

Effect of anion type and sample amount injected on retention of basic analytes. Conditions: 35/65 acetonitrile/water, 20 mM sodium acetate at pH 4.8 and 20 mM NaX ($X = \text{Cl}^-$, CF_3COO^- , ClO_4^- , PF_6^-). (A) desipramine (B) amitriptyline. Plot symbols, same as Fig. 7.

Fig. 11. Effect of retention on sample loading capacity of basic analyte. Solute, (+) alprenolol, ($\times$) doxepin, ($\square$) desipramine, ($\circ$) nortriptyline, (Δ) amitriptyline, and ($\diamond$) perphenazine. Conditions: 35/65 acetonitrile/water, 20 mM sodium acetate at pH 4.8 and (A) 20 mM NaX (X = Cl^- , CF_3COO^- , ClO_4^- , PF_6^-); (B) 0–60 mM NaClO_4 (constant ionic strength by NaCl).

Table 1

Effect of anion type on K_{ip} and k'_{max} .^a [1].

solute	k'_0 ^b	k'_{max} ^c			$K_{ip}(M^{-1})$ ^d			k'_{max}/k'_0		
		CF_3COO^-	ClO_4^-	PF_6^-	CF_3COO^-	ClO_4^-	PF_6^-	CF_3COO^-	ClO_4^-	PF_6^-
nortriptyline	6.32	43.9	56.9	89.5	$1.64_{\pm 0.04}$	$3.17_{\pm 0.56}$	$7.18_{\pm 0.56}$	6.9	9.0	14
amitriptyline	7.78	49.3	56.0	92.5	$1.65_{\pm 0.16}$	$3.93_{\pm 0.37}$	$9.15_{\pm 0.39}$	6.3	7.2	12
perphenazine	10.39	67.3	83.7	154	$1.81_{\pm 0.06}$	$3.36_{\pm 0.38}$	$5.76_{\pm 0.26}$	6.5	8.1	15

^a Chromatographic conditions: 35/65 acetonitrile/water, 20 mM sodium acetate buffer at pH 4.8 and 0–60 mM NaX, constant ionic strength maintained by sodium chloride. See Eq. 1 for definitions of parameters.

^b Retention factor of cations in the absence of anionic additives.

^c The limiting retention factor of the ion paired cation.

^d K_{ip} obtained by capillary electrophoresis with standard error in subscript.

Table 2
Effect of anion type on limiting plate count, sample loading capacity, and retention of basic drugs. ^a

anion	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	N ₀ ^b	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio
Cl ⁻	3570	1.00	2850	1.00	3920	1.00	3990	1.00	3950	1.00	3560	1.00
CF ₃ COO ⁻	3790	1.06	3010	1.06	4010	1.02	4080	1.02	3910	0.99	3640	1.02
ClO ₄ ⁻	4130	1.16	3240	1.14	4160	1.06	4150	1.04	3940	1.00	3690	1.04
PF ₆ ⁻	4060	1.14	3430	1.20	4000	1.02	3890	0.97	3740	0.95	3600	1.01
anion ^a	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	ω _{0.5} ^c	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio
Cl ⁻	6.88	1.00	7.37	1.00	5.71	1.00	4.43	1.00	4.32	1.00	5.77	1.00
CF ₃ COO ⁻	8.39	1.22	9.23	1.25	7.92	1.39	5.97	1.35	6.02	1.39	8.44	1.46
ClO ₄ ⁻	9.65	1.40	12.6	1.71	10.2	1.79	8.34	1.88	8.95	2.07	11.4	1.97
PF ₆ ⁻	14.4	2.09	24.3	3.29	17.1	3.00	14.4	3.26	17.7	4.09	23.0	3.99
anion ^a	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio
Cl ⁻	2.34	1.00	3.93	1.00	5.62	1.00	6.88	1.00	8.47	1.00	11.41	1.00
CF ₃ COO ⁻	3.05	1.30	4.98	1.27	7.25	1.29	8.81	1.28	10.66	1.26	14.52	1.27
ClO ₄ ⁻	3.93	1.68	6.46	1.65	9.41	1.67	11.50	1.67	13.83	1.63	18.32	1.61
PF ₆ ⁻	7.72	3.29	13.06	3.33	18.70	3.32	22.83	3.32	27.82	3.29	34.76	3.05

^a Conditions, same as Fig. 7. Ratios based on the relative values over chloride media.

^b Limiting plate count

^c sample loading capacity in mmol.

Table 3
Effect of perchlorate concentration on limiting plate count, sample loading capacity, and retention of basic drugs. ^a

[ClO ₄ ⁻]/(mM)	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	N ₀ ^b	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio
0	3280	1.00	2680	1.00	3760	1.00	3850	1.00	3660	1.00	3170	1.00
40	3780	1.15	3310	1.24	4020	1.07	4150	1.08	3950	1.08	3600	1.14
60	3970	1.21	3420	1.28	4090	1.09	4120	1.07	3940	1.08	3670	1.16
[ClO ₄ ⁻]/(mM) ^a	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	ω _{0.5} ^c	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio	ω _{0.5}	ω _{0.5} ratio
0	11.2	1.00	11.4	1.00	8.51	1.00	6.48	1.00	6.37	1.00	20.7	1.00
40	16.0	1.42	23.6	2.07	18.5	2.18	11.8	1.83	13.9	2.18	33.8	1.63
60	18.7	1.66	30.9	2.71	23.2	2.73	17.6	2.72	19.0	2.98	35.7	1.72
[ClO ₄ ⁻]/(mM) ^a	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio
0	2.27	1.00	3.77	1.00	5.37	1.00	6.57	1.00	8.11	1.00	10.90	1.00
40	4.23	1.87	6.90	1.83	10.17	1.89	12.36	1.88	14.77	1.82	19.59	1.80
60	4.85	2.14	7.80	2.07	11.65	2.17	14.05	2.14	16.58	2.04	22.43	2.06

^a Conditions, same as Fig. 8. Ratios based on the relative values over 0 mM of ClO₄⁻.

^b Limiting plate count

^c sample loading capacity in nmol.

Table 4

Effect of mobile phase condition on limiting plate count, sample loading capacity, and retention of basic drugs.

alprenolol	N_0^c	N_0^e ratio	$\phi_{0.5}^d$	$\phi_{0.5}^e$ ratio	k'	k'^e ratio
condition 1 <i>a</i>	3060	1.00	0.36	1.00	1.99	1.00
condition 2 <i>b</i>	3920	1.28	6.08	17.1	2.74	1.38
doxepin						
condition 1 <i>a</i>	2770	1.00	0.25	1.00	3.14	1.00
condition 2 <i>b</i>	3290	1.19	5.42	21.4	4.2	1.33
amitriptyline						
condition 1 <i>a</i>	3440	1.00	0.23	1.00	6.69	1.00
condition 2 <i>b</i>	4380	1.27	4.50	19.7	8.76	1.31
perphenazine						
condition 1 <i>a</i>	3420	1.00	0.21	1.00	6.33	1.00
condition 2 <i>b</i>	3860	1.13	7.81	36.4	7.31	1.15

a Mobile phase condition 1, 35/65 acetonitrile/water, 0.05% formic acid.

b Mobile phase condition 2, 35/65 acetonitrile/water, 0.1% formic acid with 20mM CF₃COONa.

c Limiting plate count

d sample loading capacity in nmol.

e Ratios of the values under condition 2 over condition 1.

Table 5

Effect of mobile phase condition on limiting plate count, sample loading capacity, and retention of basic drugs.

	N_0^c	N_0 ratio ^e	$\omega_{0.5}^d$	$\omega_{0.5}$ ratio ^e	k'	k' ratio ^e
alprenolol						
formate buffer ^a	3920	1.00	6.08	1.00	2.74	1.00
acetate buffer ^b	3790	0.97	8.39	1.38	3.05	1.11
amitriptyline						
formate buffer	4380	1.00	4.5	1.00	8.76	1.00
acetate buffer	3910	0.89	6.02	1.34	10.66	1.22
doxepin						
formate buffer	3290	1.00	5.42	1.00	4.2	1.00
acetate buffer	3010	0.91	9.23	1.70	4.98	1.19
perphenazine						
formate buffer	3860	1.00	7.81	1.00	7.31	1.00
acetate buffer	3640	0.94	8.44	1.08	14.52	1.99

^a Conditions, 35/65 acetonitrile/water, 20mM sodium acetate at pH 4.8 with 20 mM CF₃COONa.

^b Conditions, 35/65 acetonitrile/water, 0.1% formic acid at pH about 2.5 with 20 mM CF₃COONa.

^c Limiting plate count

^d sample loading capacity in nmol.

^e Ratios of the values under acetate buffer over formate buffer.

Table 6
Effect of anion concentration on limiting plate count, sample loading capacity, and retention of basic drugs.^a

anion /mM	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	N ₀ ^b	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio	N ₀	N ₀ ratio
Cl ⁻ /20	3570	1.00	2850	1.00	3920	1.00	3990	1.00	3950	1.00	3560	1.00
Cl ⁻ /60	3280	0.92	2680	0.94	3760	0.96	3850	0.96	3660	0.93	3170	0.89
ClO ₄ ⁻ /20	4130	1.00	3240	1.00	4160	1.00	4150	1.00	3940	1.00	3690	1.00
ClO ₄ ⁻ /60	3970	0.96	3420	1.05	4090	0.98	4120	0.99	3940	1.00	3670	1.00
anion ^a /solute	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio
Cl ⁻ /20	6.88	1.00	7.37	1.00	5.71	1.00	4.43	1.00	4.32	1.00	5.77	1.00
Cl ⁻ /60	11.2	1.63	11.4	1.55	8.51	1.49	6.48	1.46	6.37	1.48	20.7	3.59
ClO ₄ ⁻ /20	9.65	1.00	12.6	1.00	10.2	1.00	8.34	1.00	8.95	1.00	11.4	1.00
ClO ₄ ⁻ /60	18.7	1.94	30.9	2.45	23.2	2.27	17.6	2.11	19.0	2.12	35.7	3.13
anion ^a /solute	alprenolol		doxepin		desipramine		nortriptyline		amitriptyline		perphenazine	
	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio	k'	k' ratio
Cl ⁻ /20	2.34	1.00	3.93	1.00	5.62	1.00	6.88	1.00	8.47	1.00	11.41	1.00
Cl ⁻ /60	2.27	0.97	3.77	0.96	5.37	0.95	6.57	0.95	8.11	0.96	10.90	0.96
ClO ₄ ⁻ /20	3.93	1.00	6.46	1.00	9.41	1.00	11.50	1.00	13.83	1.00	18.32	1.00
ClO ₄ ⁻ /60	4.85	1.24	7.80	1.21	11.65	1.24	14.05	1.22	16.58	1.20	22.43	1.22

^a Conditions: 35/65 acetonitrile/water, 20mM sodium acetate at pH 4.8 and 20 mM or 60 mM NaX (X = Cl⁻, ClO₄⁻). Ratios based on the relative values of 60 mM over 20 mM for a fixed additive.

^b Limiting plate count

^c sample loading capacity in nmol.

Table 7

Effect of anion type on the fraction of ion pairing of the analyte. ^a

<i>α</i> _{ion pair} (%)		ion pair in the stationary phase ^b			ion pair in the mobile phase ^c		
[X ⁻]	mM	nortriptyline	amitriptyline	perphenazine	nortriptyline	amitriptyline	perphenazine
CF ₃ COO ⁻	0	0	0	0	0	0	0
CF ₃ COO ⁻	20	19	20	28	3	3	3
CF ₃ COO ⁻	40	31	34	44	6	6	7
CF ₃ COO ⁻	60	41	44	54	9	9	10
ClO ₄ ⁻	0	0	0	0	0	0	0
ClO ₄ ⁻	20	36	36	35	6	7	6
ClO ₄ ⁻	40	53	53	52	11	14	12
ClO ₄ ⁻	60	63	63	62	16	19	17
PF ₆ ⁻	0	0	0	0	0	0	0
PF ₆ ⁻	20	67	69	63	13	15	10
PF ₆ ⁻	40	80	81	77	22	27	19
PF ₆ ⁻	60	86	87	84	30	35	26

^aChromatographic conditions: same as Table 1

^bCalculated according to Eq. 9.

^cCalculated according to $\alpha_{\text{ion pair,m}} = \frac{K_{ip} [X^-]_m}{1 + K_{ip} [X^-]_m}$