The influence of organic sample solvents on the separation efficiency of basic compounds under strong cation exchange mode

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HIGHLIGHTS

- The effect of organic sample solvents in SCX mode were systematically studied.
- Organic sample solvents affect the separation efficiency in SCX mode seriously.
- Elution strength of organic solvents affect efficiency of later-eluted analyte.
- Retention of organic solvents affected efficiency of earlier-eluted analyte.
- The results can be used for the optimization of SCX methods.

GRAPHICAL ABSTRACT
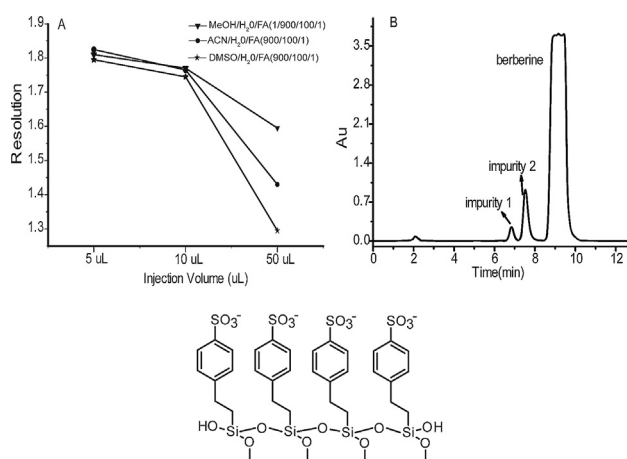
ABSTRACT

This study investigated the influence of organic sample solvents on separation efficiency of basic compounds under strong cation exchange (SCX) mode. The mixtures of acidic aqueous solution and organic solvent such as acetonitrile, ethanol, methanol and dimethyl sulfoxide (DMSO) were tested as sample solvents. For later-eluting analytes, the increase of sample solvent elution strength was responsible for the decrease of separation efficiency. Thus, sample solvents with weak elution strength could provide high separation efficiencies. For earlier-eluting analytes, the retention of organic sample solvents was the main factor affecting separation efficiency. Weakly retained solvents could provide high separation efficiency. In addition, an optimized approach was proposed to reduce the effect of organic sample solvent, in which low ionic solvent was employed as initial mobile phase in the gradient. At last, the analysis of impurities in hydrophobic drug berberine was performed. The results showed that using acidic aqueous methanol as sample solvents could provide high separation efficiency and good resolution ($R > 1.5$).

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1. Introduction

Strong cation exchange (SCX) is widely applied to separate various analytes including ions [1], proteins [2–4], peptides [5–7] and basic compounds [8–14]. Using SCX to separate basic compounds offers following advantages: (i) strong retention of polar basic compounds [12,14]; (ii) quite different separation mechanism [13] from reversed phase liquid chromatography (RPLC) [15] and (iii) high selectivity between basic compounds and non-basic compounds which can be used to selectively enrich [16] or separate basic compounds [15]. Most of basic compounds are hydrophobic and always have poor solubility in aqueous solvents. Hence organic solvent is usually needed to dissolve the basic compounds. Such organic sample solvent has been observed to cause peak broadening, distortions or even splitting of peaks [17–19].

In recent years, many reports discussed the effect of sample solvent in RPLC [17,18,20], hydrophilic interaction liquid chromatography (HILIC) [21] and normal phase liquid chromatography (NPLC) [22]. A simple way to reduce the effect of sample solvent is to dissolve the sample with the solvents which closely approximated the composition of the initial starting mobile phase and injecting with a proper volume [19], but this is not always practical. For example, for the analysis of hydrophobic basic impurities in pharmaceutical industry, a high content of organic solvent is needed due to their poor solubility in water and at the same time a large injection volume is usually necessary because of their low concentration. Although the effect of organic solvent in mobile phase under SCX mode has been extensively studied [11,12,14], the effect in sample solvent has rarely been explored. In this study, the effects of organic sample solvents on separation efficiency was systematically investigated under SCX mode. Mixtures of water and several typical organic solvents including acetonitrile, ethanol, methanol and dimethyl sulfoxide were chosen to be sample solvents and their influences on separation efficiency were investigated. Furthermore, the practical methods for improving separation efficiency were proposed.

2. Materials and methods

2.1. Chemicals

Cytosine, propranol and amitriptyline were purchased from TCI (Tokyo, Japan). Sinomenine and berberine were from J&K (Hebei, China). Acetonitrile (ACN), ethanol and methanol were obtained from Merck (Germany). Dimethyl sulfoxide (DMSO) and formic acid (FA) were obtained from J&K (Hebei, China). Acetone and naphthalene were from Kermel (Tianjin, China). All solvents were HPLC grade. Water was prepared by a Milli-Q system (Billerica, MA, USA).

2.2. Instruments

The chromatographic system consisted a 2695HPLC pump and a 2489 ultraviolet-visible detector. Datas were collected and analyzed by Empower software version 3.0. These instruments and workstations were purchased from Waters (Milford, USA). The retention of ACN, EtOH, MeOH and DMSO on XCharge SCX column was measured using a chromatographic system (Htiachi,Tokyo, Japan) which contained a 5110 pump, a 5210 auto sampler, a 5310 column oven and a 5450 refractive index (RI) detector. The XCharge SCX column (150 mm length × 4.6 mm i.d., 5 μm dia.) was purchased from Acchrom Co., Ltd. (Beijing, China), whose stationary phase was ethylbenzenesulfonic acid group [13] and synthesized by horizontal polymerization technique. The viscosity of organic solvent solutions was tested by NDJ-79 viscometer (San

Se, Shang Hai, China). The pH value was measured by an ORION pH meter (Model 686, Thermo Fisher, USA).

2.3. Sample preparation

10 mL stock solution containing 67 mg amitriptyline, 54 mg propranolol, 50.5 mg sinomenine and 40 mg cytosine was prepared. To study the effect of organic solvents, 20 μL stock solution was added into the following sample solvents: organic solvent/ water/formic acid (200/800/1, v/v/v), (500/500/1, v/v/v) or (900/ 100/1, v/v/v). To study the effect of the mixture of organic solvent and salt, 20 μL stock solution was added into sample solvents consisting of 40% ACN and 50 mM NaH_2PO_4 (pH 2.8). For each of these four sample solvent compositions, serial dilutions were made to accommodate injection volumes from 1 to 100 μL while maintaining equivalent mass loads in the linear isotherm range.

2.4. Data analysis

Separation efficiency (N) was measured according to United States Pharmacopoeia (USP): $N = 5.54 \ (t_R/W_{h/2})^2$. t_R is retention time. $W_{h/2}$ is the peak width at half-height, obtained directly by electronic integrators.

2.5. Retention of organic sample solvents

The retentions of organic sample solvents were detected by RI detector [18,23]. The retentions of 90% ACN, 90% ethanol, 90% methanol, and 90% DMSO with different injection volumes were measured. The mobile phase was ACN/100 mM NaH_2PO_4 (pH 2.8)/ water (40/50/10, v/v/v). In addition, a solution which has the same composition with mobile phase was injected to obtain system peaks.

3. Results and discussion

According to the previous observations [12,24], the hydrophobic interaction has an influence on the total retention of basic compounds under SCX mode. However, organic solvent is always inevitably used to assist the solubilization of basic compounds. Herein the influence of organic sample solvents on the separation efficiency of basic compounds was explored by using four common organic solvents including ACN, EtOH, MeOH and DMSO. Four basic compounds with various pKa and log P (their chemical structures are illustrated in Fig. 1) were used as model sample to evaluate the effect of organic sample solvents. Because the retention of charged analyte was related to its ionization state and sample solvents which have not been acidified will lead to peak distortion and efficiency decrease of the analytes under SCX mode (Figs. S1–S4). Thus 0.0265 mol L^{-1} formic acid was applied to acidify sample solvents and ionize analytes.

3.1. Effect of organic solvent type

Acetonitrile is mostly used in RPLC and SCX system as it provides symmetric peak shape and good selectivity. Ethanol and methanol are usually applied to extract compounds from natural products. DMSO is also a commonly used solvent due to its good ability to dissolve a wide variety of analytes at high concentration [21,25]. Therefore, these four organic solvents mixed with acidic aqueous solution, which organic solvent/water/formic acid is 900/ 100/1 (v/v/v), were used as sample solvent and their effects on separation efficiency under SCX mode were investigated (Fig. 2(A)).

When 90% acetonitrile is used as sample solvent, a loss of 5%, 9%, 19% and 21% of efficiency is observed for cytosine, sinomenine,
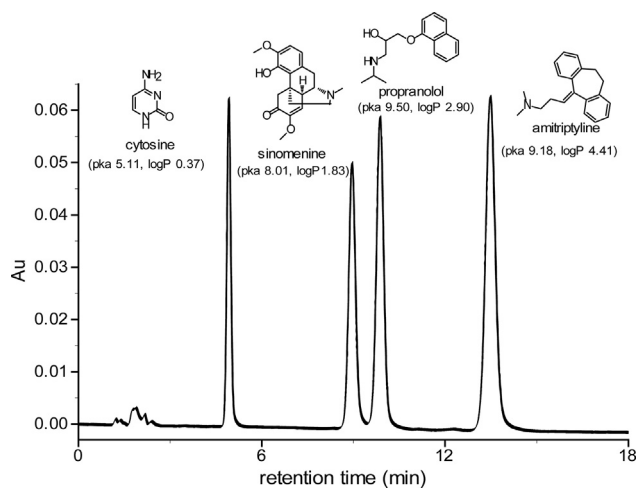
Fig. 1. The chromatogram of cytosine, sinomenine, propranolol and amitriptyline. Mobile phase was ACN/100 mM NaH$_2$PO$_4$ (pH 2.8)/water (40/50/10, v/v/v). The flow rate was 1.0 mL min^{-1}.

Fig. 3. Plot of k vs. 1/[C_{salt}] on SCX column. A: ACN, B: 100 mM NaH$_2$PO$_4$ (pH 2.8), C: water; mobile phase was: 40% A, 20%/30%/40%/50%/60% B. The flow rate was 1.0 mL min^{-1}.

propranolol and amitriptyline respectively, compared to which of water/formic acid (1000/1, v/v) used as sample solvent. It is interesting to note that 90% acetonitrile sample solvent had a more dramatic influence on later-eluting peaks, which is much different from that of RPLC mode [19,20]. In RPLC mode, a high content of acetonitrile as sample solvent, the efficiency of weakly retained analyte decreases more obviously than that of strongly retained
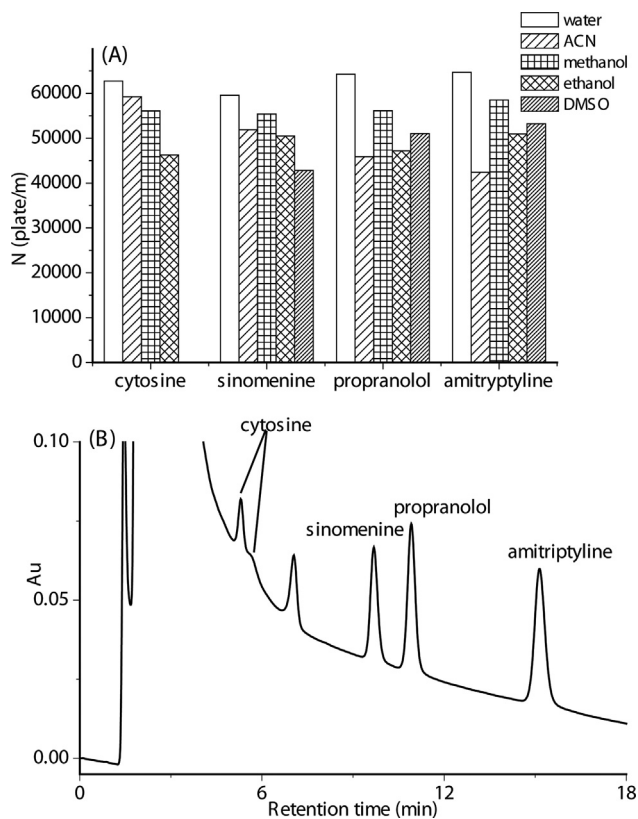

Fig. 2. (A) The influence of organic solvent type on the separation efficiency of basic compounds. Sample solvents were organic solvents/water/formic acid (900/100/1, v/v/v) and water/formic acid (1000/1, v/v). Separation efficiency was not reported for cytosine in 90% DMSO due to the distorted peak shape. (B) Chromatogram of basic compounds when 90% DMSO was used as sample solvent. The injection volume was 100 μL. Mobile phase was ACN/100 mM NaH$_2$PO$_4$ (pH 2.8)/water (40/50/10, v/v/v). The flow rate was 1.0 mL min^{-1}.
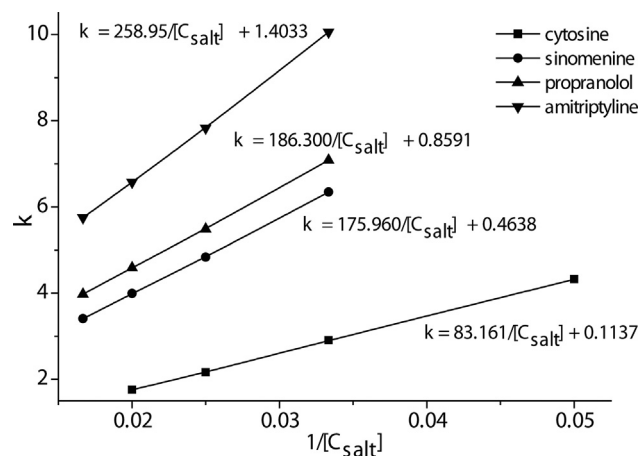
ones. According to our previous study [12] and the equation ($k = k_{RP} + B_{IEX}/[C^+]_m$) developed by Luo et al. [24], each basic compound had an B_{IEX} value. The compound which has larger B_{IEX} value usually has longer retention under SCX mode (Fig. 3). With increasing organic solvent content, the change of retention for analyte which has a large B_{IEX} value will be more dramatic [12]. If the organic solvent content in sample solvent is higher than in the mobile phase, the migration velocity of analyte which has larger B_{IEX} value in the sample solvent is much larger than in the mobile phase. As the leading front of the analyte band moves faster than its rear front, the analyte band is stretched and results in peak broadening. For analyte which has a small B_{IEX} value, peak broadening is not obvious, since the difference of its migration velocity in sample solvent and in mobile phase is minor.

Using 90% DMSO as sample solvent, the separation efficiencies of sinomenine, propranolol and amitriptyline are decreased by 16%, 13% and 9%, respectively. The change of cytosine efficiency could not be measured, since peak splitting is observed under this condition (Fig. 2(B)).Thus the influence of DMSO on weakly retained basic compound is more dramatic than that of strongly retained one, which is not consistent with the influence of acetonitrile. Upon further study, this behavior is related to the retention of organic solvent. Loeser and Drumm [18] has been reported that the sample solvent produced peak broadening effects for the analytes which eluted immediately after the sample solvent under RPLC mode, and the most noticeable loss of separation efficiency for the analyte is observed when it is eluted very close to the injection solvent plug. As shown in Table 1 (the solvent injection chromatograms have been shown in Fig. S5), the retention of organic solvents increase in the order: methanol ≈ acetonitrile < ethanol < DMSO. DMSO has the longest retention time (4.04 min). For weakly retained base (cytosine), whose

Table 1
The retention time (min) of organic sample solvents with different injection volumes.

Volume/solvent	1 μL	10 μL	50 μL	100 μL
MeOH	2.080	2.092	2.162	2.190
ACN	2.256	2.254	2.301	2.376
EtOH	2.488	2.571	2.800	3.080
DMSO	2.574	2.596	3.561	4.043

Organic sample solventsconsisted of organic solvent/ water/formic acid (900/100/1, v/v/v). The injection volume was 1, 10, 50 and100 μL, respectively. The mobile phase was ACN/ 100 mM NaH$_2$PO$_4$ (pH 2.8)/water (40/50/10, v/v/v).

retention time is 5.25 min, the loss of separation efficiencies increase in the order: methanol ≈ acetonitrile < ethanol < DMSO. This agrees well with the order of retention of organic solvents. Therefore, the strong retention of sample solvent is the main factor for the decreasing efficiency of cytosine. For strongly retained basic compounds, they are less affected by strongly retained sample solvent, since sample solvent plug has been diffused by mobile phase at the time the analyte band elutes.

When 90% methanol is used as the sample solvent, 12%, 4%, 10% and 6% of separation efficiencies was lost for cytosine, sinomenine, propranolol and amitriptyline, respectively. In comparison, the loss of separation efficiency for 90% ethanol solvent is 21%, 13%, 20% and 15% for cytosine, sinomenine, propranolol and amitriptyline, respectively. The decreases of separation efficiencies for all compounds are more pronounced using ethanol than methanol. As shown in Table 2, the elution strength of ethanol is stronger than methanol. Furthermore, the retention of ethanol is larger than methanol. Thus, the influence of ethanol on separation efficiency is more noticeable than that of methanol.

As a conclusion, the elution strength and retention of organic solvent contributes to the change of analyte separation efficiency. In this study, the ability to elute naphthalene was tested to demonstrate the elution strength of organic solvents and the elution strength increases in the order (Table 2): methanol < DMSO < ethanol < acetonitrile. For strongly retained bases, the influence of organic solvent elution strength on separation efficiency is more significant than that of organic solvent retention. For example, the loss of efficiency of amitriptyline increases in the order: methanol < DMSO < ethanol < acetonitrile, which agrees well with the elution strength of these solvents. Nevertheless,

Table 2
The strength of organic solvents.

	ACN	EtOH	DMSO	MeOH
$t_{R(naphthalene)}$/min	7.639	9.471	9.863	12.477

$t_{R(naphthalene)}$: the retention time of naphthalene on SCX column. The mobile phase was organic solvents/water (10/90, v/v). The flow rate was 1.0 mL min^{-1}.

for weakly retained bases, the strong retention of sample solvent is the main factor influencing efficiency.

3.2. The effect of injection solvent volume

The effect of sample volume was also investigated, as shown in Fig. 4. There is no distortion in the separation efficiencies of all analytes when the injection volume is 1 μL. However, with increasing injection volume, the separation efficiency decreases. For strongly retained compounds (e.g., amitriptyline), the decrease of efficiency is the most obvious for 90% ACN solvent, which is consistent with the change of the elution strength of sample solvents. Therefore, the increase of sample solvent elution strength is the main reason for the decrease of separation efficiency of strongly retained compounds. For weakly retained compounds (e.g., cytosine), the decrease of efficiency is much less for 90% ACN. As discussed in Section 3.1, the increase of sample solvent retention is the main factor for the efficiency decrease of earlier-eluting analyte. The retentions of all selected organic solvents obtained with different injection volumes are listed in Table 1. The change of the retention time for model organic solvents increases in the order: methanol ≈ acetonitrile < ethanol < DMSO. As a result, the loss of cytosine efficiency is in the same order.
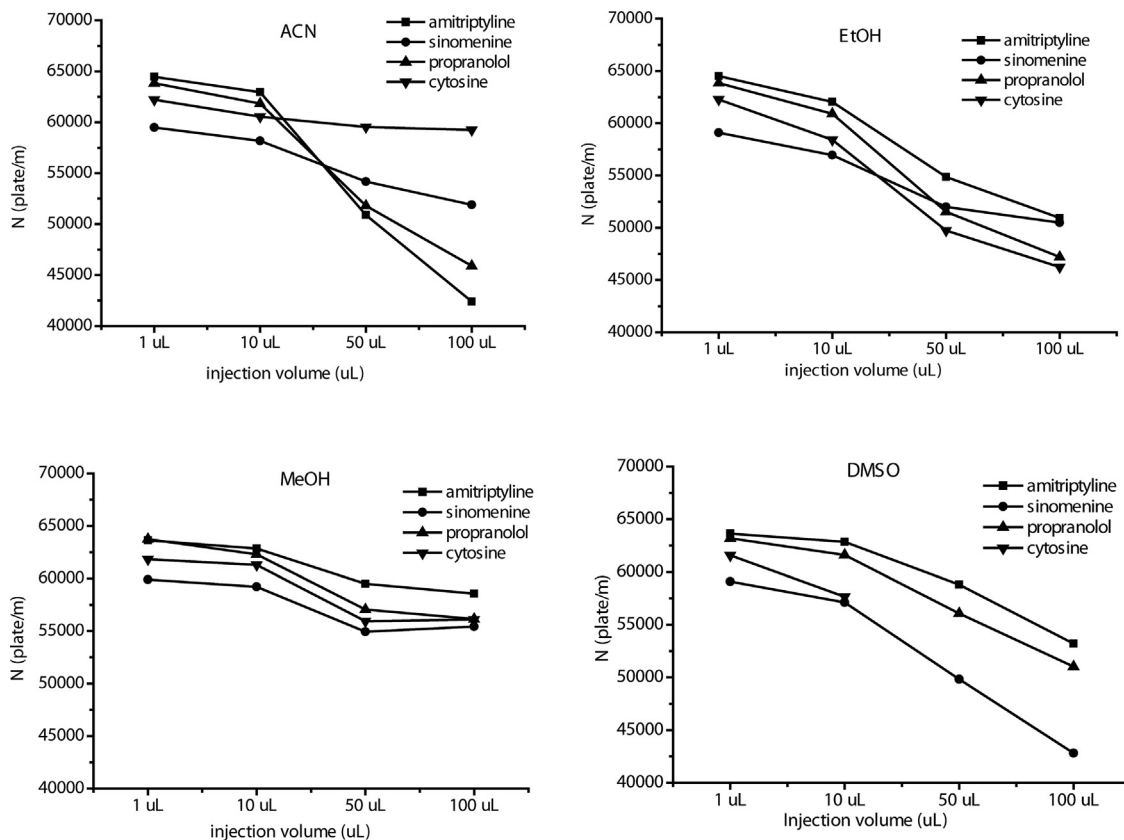

Fig. 4. The separation efficiency of basic compounds with various injection volumes. Sample solvents were organic solvents/water/formic acid (900/100/1, v/v/v). The injection volume was 1, 10, 50 and 100 μL, respectively. Separation efficiencies were not reported for cytosine in 90% DMSO with injection volume 50 μL and 100 μL due to the distorted peak shapes. Mobile phase was ACN/100 mM NaH$_2$PO$_4$ (pH 2.8)/water (40/50/10, v/v/v). The flow rate was 1.0 mL min^{-1}.
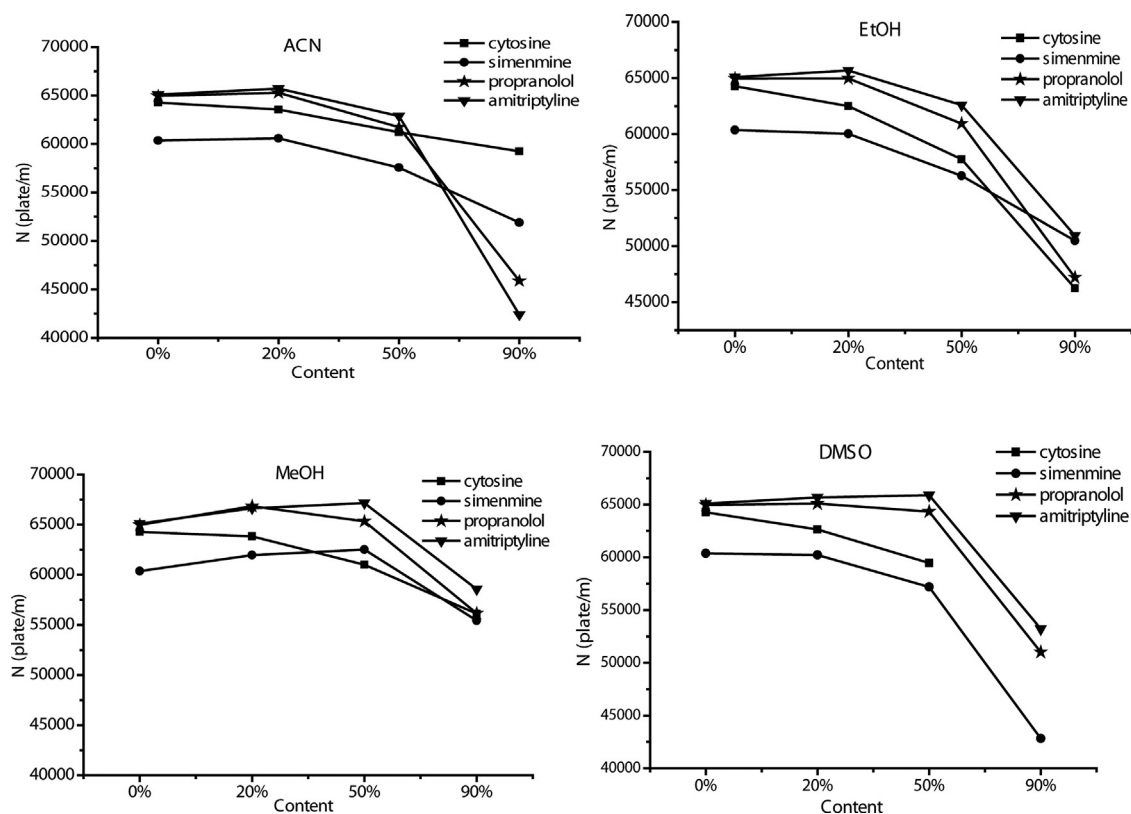
Fig. 5. The separation efficiency of basic compounds using various contents organic solvent as sample solvent. All of solvent contained 0.0265 mol L^{-1} formic acid. The injection volume was 100 μL. Value was not reported for cytosine in 90% DMSO with injection volume 100 μL due to the distorted peak shape. Mobile phase was ACN/100 mM NaH$_2$PO$_4$ (pH 2.8)/water (40/50/10, v/v/v). The flow rate was 1.0 mL min^{-1}.

3.3. Effect of organic solvent content in the sample solution

High content organic solvent is usually applied to improve the solubility of samples, especially for hydrophobic samples. Fig. 5 demonstrates the effect of organic solvent content on separation efficiency. It can be seen that the separation efficiency decreases with the increase of the content of organic solvent in the sample solution. As similar to Section 3.2, for strongly retained compounds (e.g., amitriptyline), the decrease of efficiency is more significant with the change of ACN content. The increase of the organic solvent content leads to an increase of the sample solvent elution strength and the loss of analyte separation efficiency. For weakly retained compound (cytosine), the decrease of efficiency is the most obvious by changing the content of DMSO. Thus the retention time of organic sample solvents is the main factor influencing separation efficiency of weakly retained compound.

In addition, the viscosity of sample solvent is usually reported as one factor for the separation efficiency decrease of earlier-eluting analyte [17]. According to viscosity fingering theory, peak distortion increases as the difference in the viscosity of mobile phase and sample dilution increases. The viscosity of 90% DMSO and 50% DMSO are measured to be 3.34 mPa s^{-1} and 3.61 mPa s^{-1} respectively (Fig. S6). Based on viscosity considerations, peak distortion would be improved when 90% DMSO is used as sample solvent instead of 50% DMSO. Nevertheless, the separation efficiencies of earlier-eluting analytes obtained using 90% DMSO are lower than using 50% DMSO, and the most notably the peak splitting of cytosine was observed. These results suggest that the increase of sample solvent viscosity is not the main factor for the decrease of analyte efficiency.

3.4. Effect of the mixture of organic solvent and aqueous salt

Both acid and buffer solution can be applied to ionize basic compounds. In above sections, formic acid was used to acidify analytes. In this section, sample solvent was acidified via formic acid and buffer solution, respectively. Fig. 6 shows the
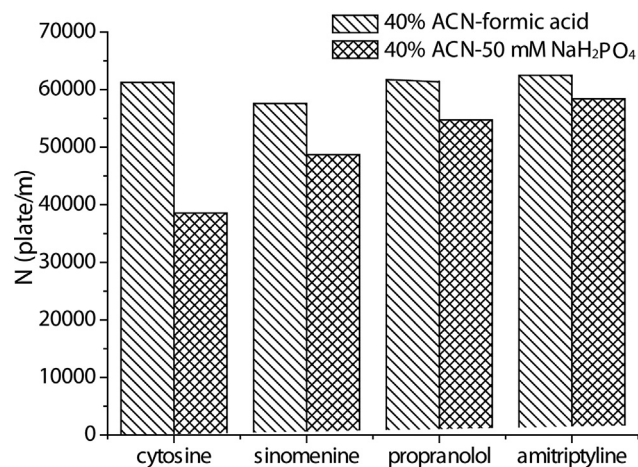

Fig. 6. The effect of the mixture of organic solvent and aqueous salt. The sample solvent is 40% ACN-formic acid (ACN/water/formic acid: 400/600/1, v/v/v) or 40% ACN-50 mM NaH$_2$PO$_4$ (ACN/100 mM NaH$_2$PO$_4$, pH 2.8/water: 400/500/100, v/v/v). The injection volume was 100 μL. Mobile phase was ACN/100 mM NaH$_2$PO$_4$ (pH 2.8)/water (40/50/10, v/v/v). The flow rate was 1.0 mL min^{-1}.
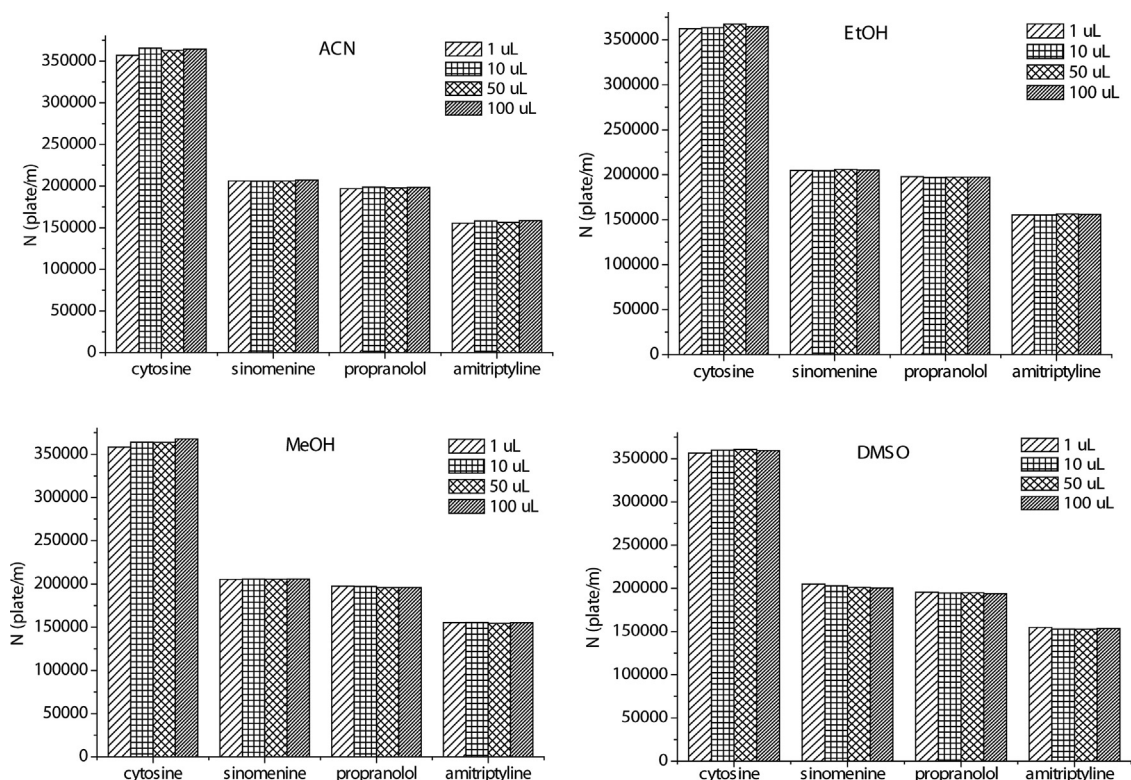
Fig. 7. The separation efficiency of basic compounds using initial mobile phase without salts. Sample solvents were organic solvents/water/formic acid (900/100/1, v/v/v). The injection volume was 1, 10, 50 and 100 μL, respectively. (A) ACN, (B) 100 mM NaH$_2$PO$_4$ (pH 2.8), (C) water(containing 0.0265 mol L^{-1} formic acid). Mobile phase were: 0–5 min, A/B/C: 40/0/60 (v/v); 5–10 min, A/B/C: 40/0/60 → 40/50/10 (v/v/v). The flow rate was 1.0 mL min^{-1}.

corresponding separation efficiencies of four basic compounds when the sample solvent is 40% ACN-formic acid (ACN/water/formic acid: 400/600/1, v/v/v) or 40% ACN-50 mM NaH$_2$PO$_4$ (ACN/100 mM NaH$_2$PO$_4$, pH 2.8/water: 400/500/100, v/v/v). As shown, high separation efficiency can be obtained for all compounds dissolved in 40% ACN-formic acid. However, when 40% ACN-50 mM NaH$_2$PO$_4$ is used to diluted samples, the separation efficiencies of all analytes are sharply decreased. Compared to using 40% ACN-formic acid as sample solvent, the separation efficiencies of cytosine, sinomenine, propranolol and amitriptyline are decreased 37%, 15%, 11% and 7%, respectively. Thus, improved separation efficiency under SCX mode can be obtained by using acid acidified sample solvents, especially for weakly retained compounds.

3.5. Practical methods for separation efficiency improvement

Sometimes, samples can't be avoided to be dissolved in high content organic solvent or salt, and injected with a large volume. Thus, it is necessary to develop a practical method used in such cases with much less loss of separation efficiency. Under SCX mode, if the salt concentration is low enough, the analytes can't be effectively eluted since salt is required for the elution of analytes from ion exchange sites. As a result, the analytes would be enriched in the column head while the sample solvents were eluted, and thus the effect of the solvent is minimized. Based on such idea, the mobile phase condition chosen was as follows. 40% ACN, 0–5 min and 40% ACN, 0–50 mM NaH$_2$PO$_4$ (pH 3.2), 5–10 min. (Note, there is no salt in the initial starting moble phase.) As shown in Fig. 7, the
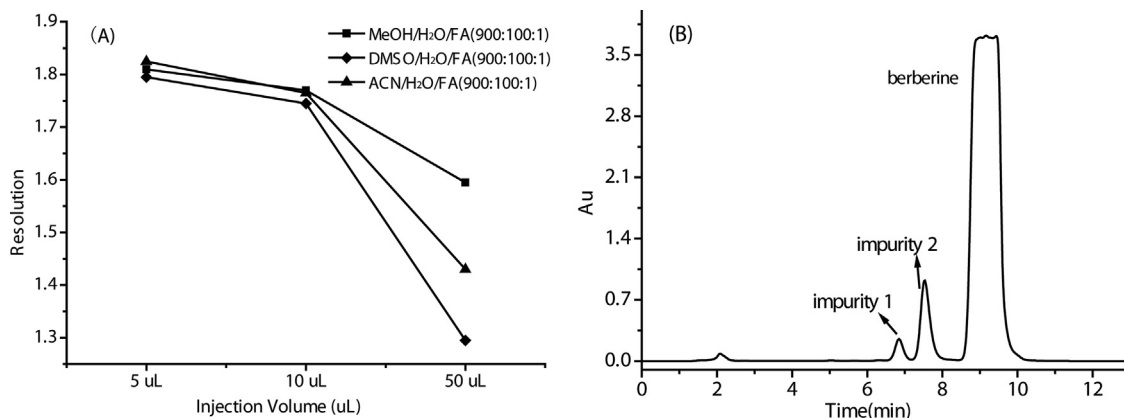

Fig. 8. (A) The influence of organic sample solvents on the resolution of impurities of berberine. Sample solvents were organic solvent/water/formic acid (900/100/1, v/v/v). The injection volume was 5, 10 and 50 μL, respectively; (B) chromatogram for the analysis of berberine. Sample solvent was MeOH/water/formic acid (900/100/1, v/v/v). The injection volume was 50 μL. Mobile phase was ACN/100 mM NaH$_2$PO$_4$ (pH 2.8) (50/50, v/v). The flow rate was 1.0 mL min^{-1}.

negative effect of organic solvent has been greatly improved. Even if the content of organic solvent in sample solvent is as high as 90% and injected sample volume is as large as 100 μL, the change of efficiency is minor for all compounds.

3.6. Application

The impurities in drug may have different bioactivities and sometimes are even toxic. Therefore, the analysis of impurities is indispensable in the pharmaceutical industry. Berberine is an important drug which has been tested and used successfully in experiment [26,27] and human diabetes mellitus [28,29]. Organic solvent (organic solvent/H_2O/formic acid, 900/100/1, v/v/v) including acetonitrile, DMSO and methanol are utilized for sample dilution, since they tended to solubilize berberine readily. To determine or purify these impurities, high injection volume is needed. It can be seen in Fig. 8A, the resolution of these two main impurities decreases as the injection volume increases, especially for the sample which diluted in DMSO and acetonitrile. Therefore, the sample solvent with long retention and high elution strength has a more dramatic influence on peak shape. Dissolving samples in acidic aqueous methanol, the solubility of samples can be enhanced and good resolution ($R > 1.5$) can be maintained (Fig. 8B).

4. Conclusion

The effects of organic sample solvents on separation efficiency under SCX mode were investigated systematically by diluting basic compounds with various organic solvents. The effects of organic solvents, including acetonitrile, ethanol, methanol and DMSO, were initially evaluated with basic compounds under SCX mode. The results indicated the elution strength and retention of sample solvent accounted for the loss of separation efficiency. For strongly retained analytes, the separation efficiency can be improved by diluting samples with weak elution strength organic solvent, such as methanol. For weakly retained analytes, high separation efficiency can be obtained by using weakly retained solvent, such as acetonitrile and methanol. If choosing sample solvent was not an option, the influence of organic solvent can be improved by using starting mobile phase which did not contain salt. The results obtained here can be used to optimize SCX methods and to explain the potential retention mechanisms under SCX mode.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.12.057.

References

[1] A.V. Pirogov, M.V. Chernova, D.S. Nemtseva, O.A. Shpigun, Sulfonated and sulfoacylated poly(styrene-divinylbenzene)copolymers as packing materials for cation chromatography, Anal. Bioanal. Chem. 376 (2003) 745–752.

[2] S. Grotefend, L. Kaminski, S. Wroblewitz, S. El Deeb, N. Kuehn, S. Reichl, M. Limberger, S. Watt, H. Waetzig, Protein quantitation using various modes of high performance liquid chromatography, J. Pharm. Biomed. Anal. 71 (2012) 127–138.

[3] R.P.W. Kong, S.O. Siu, S.S.M. Lee, C. Lo, I.K. Chu, Development of online high-/low-pH reversed-phase-reversed-phase two-dimensional liquid chromatography for shotgun proteomics: a reversed-phase-strong cation exchange-reversed-phase approach, J. Chromatogr. A 1218 (2011) 3681–3688.

[4] E. Machtejevas, H. John, K. Wagner, L. Standker, G. Marko-Varga, W.G. Forssmann, R. Bischoff, K.K. Unger, Automated multi-dimensional liquid chromatography: sample preparation and identification of peptides from human blood filtrate, J. Chromatogr. B 803 (2004) 121–130.

[5] M. Gao, W. Yu, Y. Zhang, G. Yan, C. Deng, P. Yang, X. Zhang, Integrated strong cation exchange/capillary reversed-phase liquid chromatography/on-target digestion coupled with mass spectrometry for identification of intact human liver tissue proteins, Analyst 133 (2008) 1261–1267.

[6] M.L. Hennrich, H.W.P. van den Toorn, V. Groenewold, A.J.R. Heck, S. Mohammed, Ultra acidic strong cation exchange enabling the efficient enrichment of basic phosphopeptides, Anal. Chem. 84 (2012) 1804–1808.

[7] A. Voitl, A. Butte, M. Morbidelli, Behavior of human serum albumin on strong cation exchange resins: I. Experimental analysis, J. Chromatogr. A 1217 (2010) 5484–5491.

[8] K. Croes, P.T. McCarthy, R.J. Flanagan, HPLC of basic drugs and quaternary ammonium-compounds on microparticulate strong cation-exchange materials using methanolic or aqueous-methanol eluents containing an ionic modifier, J. Chromatogr. A 693 (1995) 289–306.

[9] R.J. Flanagan, E.J. Harvey, E.P. Spencer, HPLC of basic drugs on microparticulate strong cation-exchange materials – a review, Forensic Sci. Int. 121 (2001) 97–102.

[10] P.E. Morgan, M. Hanna-Brown, R.J. Flanagan, Analysis of basic drugs: a comparison of two different strong cation exchange-modified LC packings, Chromatographia 72 (2010) 15–22.

[11] P.E. Morgan, V. Manwaring, R.J. Flanagan, HPLC of basic drugs using non-aqueous ionic eluents: evaluation of a 3 μm strong cation-exchange material, Biomed. Chromatogr. 24 (2010) 318–323.

[12] Z. Long, Z. Guo, X. Xue, X. Zhang, L. Nordahl, X. Liang, Selective separation and purification of highly polar basic compounds using a silica-based strong cation exchange stationary phase, Anal. Chim. Acta 804 (2013) 304–312.

[13] Z. Long, C. Wang, Z. Guo, X. Zhang, L. Nordahl, X. Liang, Strong cation exchange column allow for symmetrical peak shape and increased sample loading in the separation of basic compounds, J. Chromatogr. A 1256 (2012) 67–71.

[14] H. Luo, L. Ma, C. Paek, P.W. Carr, Application of silica-based hyper-crosslinked sulfonate-modified reversed stationary phases for separating highly hydrophilic basic compounds, J. Chromatogr. A 1202 (2008) 8–18.

[15] Z. Long, Z. Guo, X. Xue, X. Zhang, X. Liang, Two-dimensional strong cation exchange/positively charged reversed-phase liquid chromatography for alkaloid analysis and purification, J. Sep. Sci. 36 (2013) 3845–3852.

[16] Z. Long, C. Wang, Z. Guo, X. Zhang, L. Nordahl, J. Zeng, J. Zeng, X. Liang, A non-aqueous solid phase extraction method for alkaloid enrichment and its application in the determination of hyoscyamine and scopolamine, Analyst 137 (2012) 1451–1457.

[17] C.B. Castells, R.C. Castells, Peak distortion in reversed-phase liquid chromatography as a consequence of viscosity differences between sample solvent and mobile phase, J. Chromatogr. A 805 (1998) 55–61.

[18] E. Loeser, P. Drumm, Using strong injection solvents with 100% aqueous mobile phase in RP-LC, J. Sep. Sci. 29 (2006) 2847–2852.

[19] E.S. Kozlowski, R.A. Dalterlo, Analyte solvent and injection volume as variables affecting method development in semipreparative reversed-phase liquid chromatography, J. Sep. Sci. 30 (2007) 2286–2292.

[20] J. Layne, T. Farcas, I. Rustamov, F. Ahmed, Volume-load capacity in fast-gradient liquid chromatography – effect of sample solvent composition and injection volume on chromatographic performance, J. Chromatogr. A 913 (2001) 233–242.

[21] J. Ruta, S. Rudaz, D.V. McCalley, J.-L. Veuthey, D. Guillarme, A systematic investigation of the effect of sample diluent on peak shape in hydrophilic interaction liquid chromatography, J. Chromatogr. A 1217 (2010) 8230–8240.

[22] P. Guinebault, M. Broquaire, Large-volume injection of samples dissolved in a noneluting solvent – application to the determination of antipyrine using normal-phase high-performance liquid-chromatography, J. Chromatogr. 217 (1981) 509–522.

[23] P. Petersson, P. Forssen, L. Edstrom, F. Samie, S. Tatterton, A. Clarke, T. Fornstedt, Why ultra high performance liquid chromatography produces more tailing peaks than high performance liquid chromatography, why it does not matter and how it can be addressed, J. Chromatogr. A 1218 (2011) 6914–6921.

[24] H. Luo, L. Ma, Y. Zhang, P.W. Carr, Synthesis and characterization of silica-based hyper-crosslinked sulfonate-modified reversed stationary phases, J. Chromatogr. A 1182 (2008) 41–55.

[25] K.V. Balakin, N.P. Savchuk, I.V. Tetko, In silico approaches to prediction of aqueous and DMSO solubility of drug-like compounds: Trends, problems and solutions, Curr. Med. Chem. 13 (2006) 223–241.

[26] Y. Wang, T. Campbell, B. Perry, C. Beaurepaire, L. Qin, Hypoglycemic and insulin-sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats, Metab.: Clin. Exp. 60 (2010) 298–305.

[27] C. Wang, J. Lia, X. Lv, M. Zhanga, Y. Song, L. Chen, Y. Liu, Ameliorative effect of berberine on endothelial dysfunction in diabetic rats induced by high-fat diet and streptozotocin, Eur. J. Pharmacol. 620 (2009) 131–137.

[28] Y. Gu, Y. Zhang, X. Shi, X. Li, J. Hong, J. Chen, W. Gu, X. Lu, G. Xu, G. Ning, Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics, Talanta 81 (2010) 766–772.

[29] J.-M. Wang, Z. Yang, M.G. Xu, L. Chen, Y. Wang, C. Su, J. Tao, Berberine-induced decline in circulating CD31+/CD42- microparticles is associated with improvement of endothelial function in humans, Eur. J. Pharmacol. 614 (2009) 77–83.