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Importance of injection solution composition for LC–MS–MS methods

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Abstract

For the first time, the influence of the injection solution composition on the quality of LC–MS–MS methods, in terms of column efficiency and peak shape, was systematically investigated. Various types of compounds, including polar ionic acidic, polar ionic basic and non-polar neutral compounds, were prepared in different solutions ranging from 100% water to 100% acetonitrile. Different volumes of these solutions were injected onto either C_{18} or silica columns connected to tandem mass spectrometry. The mobile phases consisted of acetonitrile, water, and small amounts of volatile acid or buffer. On silica columns, the influence of injection solution on the peak shape and column efficiency was straightforward. The sharpest peaks and the highest column efficiency were obtained with 100% acetonitrile as the injection solvent. On C_{18} columns, this type of influence was less clear due to the dual retention mechanism of the bonded phase and of the residual silanol groups. On C_{18} column, retention due to residual silanol groups was significant even with a mobile phase containing less than 50% acetonitrile. Poor peak shape was observed when the injection solution had a stronger eluting strength than mobile phase, particularly for early eluting peaks.

Keywords: Injection solution; LC–MS–MS

1. Introduction

Columns of smaller dimension (50 mm × 2 or 3 mm, ID) have been frequently used for liquid chromatography with tandem mass spectrometric detection (LC–MS–MS) methods for analysis of compounds in biological fluid [1]. Lengthy and mandatory chromatographic separation of analytes from the endogenous interference has been essentially replaced by shorter run time, thanks to the extraordinary selectivity provided by the tandem mass spectrometry. In order to support ever-increased drug discovery and development speed, LC–MS–MS methods of faster separation time, better sensitivity and higher reliability have become the daily routine for many bioanalytical chemists. The typical run time for bioanalytical methods has decreased from >20 min for conventional LC methods to <5 min for most of the LC–MS–MS methods. The capacity factor (k'),

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which is the indication for the magnitude of the analyte on-column retention, has decreased from typically 5–10 for conventional LC methods to 2–4 for LC–MS–MS methods. In comparison with the conventional column (250×4.6 mm^2, ID), columns of much smaller dimension can provide such advantages and therefore often become the first of choices for most LC–MS–MS practitioner. In addition, the smaller columns have much lower dead volume and enable, in a relatively short time period, the resolution of analytes from most endogenous matrix compounds, which elute at or near the dead volume. Such resolution is essential for avoiding detrimental matrix effects [2]. However, because of much lower quantities of the packing material in the smaller column and because of lower retention of analytes on the column, it is more likely that chromatography can be disturbed, particularly by the mismatch of the elution strength between the injection solution and the mobile phase. The theoretical consideration of effects of injection solution to peak shape and chromatography efficiency for conventional reversed-phase LC columns has been studied by Cheng et al. [3]. The maximum chromatography efficiency was achieved by using the injection solution of the weakest elution strength. Analytes were initially stacked on the top of the analytical column upon injection and were then eluted by the mobile phase. This theoretical study was very useful for selecting an appropriate injection solution under well-defined reversed-phase chromatographic conditions. To the best of our knowledge, reports of systematic studies that investigated effects of injection solution to the quality of LC–MS–MS method have not appeared. A brief survey of literature indicated that mobile phases were used as the injection solution by most of the LC–MS–MS practitioners [4–8]. One group [9] as well as the authors [10–13] used weaker solvents as the injection solution.

In this report, we described a systematic investigation of the influence of the injection solution and its volume on the peak shape and column efficiency for fast LC–MS–MS. This knowledge can be used for the LC–MS–MS users to drive for better sensitivity, better selectivity, and more reliable quantitation.

2. Experimental

2.1. Chemicals and reagents

2-Thiophenecarboxylic acid (2-TCA, purity 99%) and 3-methyl-2-thiophenecarboxylic acid (3-MCA, purity 98%) were from Acros Organics (Pittsburgh, PA). 2-Thiopheneacetic acid (2-TAA, purity 98%) was from Aldrich (Milwaukee, WI). Nicotine (NIC, purity 99%) and cotinine (COT, purity 98%) were from Sigma (St. Louis, MO). Albuterol (ALB, purity 99%), bamethan (BAM) sulfate salt (purity 99%), dexamethasone (DEX, purity 99.9%) and beclomethasone (BEC, purity 99.7%) were also from Sigma. Formic acid, ammonium acetate, water, and acetonitrile, all with HPLC grade were from Fisher (St. Louis, MO).

2.2. LC-tandem mass spectrometry method

The LC–MS–MS system consisted of a Shimadzu series 10ADVP HPLC system (Kyoto, Japan), and Perkin Elmer Sciex API-3000 tandem mass spectrometer detectors with Turbo Ion Spray interface (Toronto, Canada). Multiple reaction mode (MRM) sensitivities were optimized by testing on an infusion of 0.1 μg ml^{-1} each of the analytes in a mixture of methanol and water (50:50, v/v). Analytes were dissolved in the mobile phases and injected onto silica or C_{18} analytical columns. Silica or C_{18} columns of 50 mm $\times$ 2 or 3 mm ID, 5 μm, all from Keystone Scientific (Bellefonte, PA) were used. The columns were maintained at ambient temperature. Positive or negative ions were monitored in the MRM mode when acidic or neutral mobile phases were used respectively. For basic amine analytes, mobile phases contained acetonitrile, water and formic acid. For acidic analytes, mobile phases were acetonitrile, water and 5 mM ammonium acetate. Once the mobile phases were selected, analytes in various solvents were injected onto the column to select injection solvent. The chromatographic conditions are shown in the figure legend.

The capacity factor (k') was calculated as $(t_R - t_O)/t_O$ where t_R is the retention of the peak. The USP plate count (N) was measured as 5.54 $(t_R/W_{0.5})^2$ where $W_{0.5}$ is the peak width at the half of

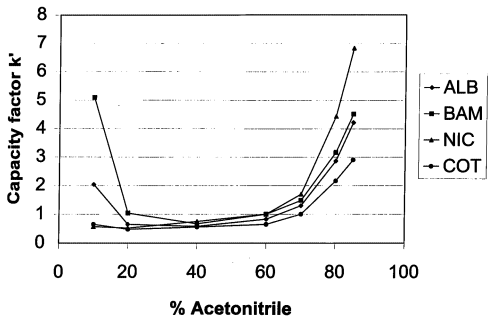

Fig. 1. *Retention of polar ionic basic compounds on a reversed-phase column*: Column: Hypersil BDS C_{18}, 50×2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water–formic acid [x:(100 − x):0.2, v/v/v); flow rate:0.5 ml min^{-1}; injection solution: corresponding mobile phase; injection volume:2 μl.

the peak height [14]. This calculation assumes Gaussian (symmetrical) peaks. For peaks with severe tailing, the Foley and Dorsey equation $N = 41.7(t_R/W_{0.1})^2/(B/A + 1.25)$ should be used, where $W_{0.1}$ is the peak width at 10% peak height and B/A is the asymmetry factor measured at the 10% peak height [15]. Foley and Dorsey equation gave slightly lower N numbers (about 10–30% lower) than USP equation for all analytes in this study with an exception of NIC (200% lower). The tailing for the NIC peak is severe while peaks of other analytes are symmetric ($B/A < 1.3$). Since the injection solution composition did not change the peak tailing significantly, the USP equation was used throughout the study.

The following positive ions were monitored:

ALB: 240 → 148; BAM: 210 → 136; NIC: 163 → 84; COT: 177 → 80; DEX: 393 → 373; BEC: 409 → 391.

The following negative ions were monitored:

3-MCA: 141 → 97; 2-TAA: 141 → 97; 2-TCA: 127 → 83.

3. Results and discussion

3.1. Chromatography retention mechanism

It is paramount to determine the retention mechanism before choosing an injection solution since the retention mechanism determines whether water or organic solvent is the stronger elution solvent. On reversed-phase LC, water is a weaker elution solvent and organic solvent is a stronger elution solvent. Conversely, on normal-phase LC, water is a stronger elution solvent and organic solvent is a weaker elution solvent. The designation 'reversed-phase' C_{18} column can sometimes be misleading and the retention mechanism on C_{18} column can be complicated. For NIC and COT, the normal phase retention mechanism can be observed at as low as 20% organic solvent in the mobile phase as shown in Fig. 1. The capacity factors (k') and plate counts (N) were summarized in Table 1. Better column efficiency, in terms of plate count, was observed when the retention is under either predominate reversed-phase mechanism (10% acetonitrile in mobile phase) or predominate normal phase mechanism (> 60% acetonitrile in mobile phase). When both reten-

Table 1
Influence of concentration of acetonitrile in the mobile phase on capacity factor (k') and plate count (N)

(%) Acetonitrile in mobile phase	ALB		BAM		NIC		COT	
	k'	N	k'	N	k'	N	k'	N
10	2.04	600	5.09	936	0.57	166	0.65	209
20	0.65	202	1.04	318	0.52	123	0.48	163
40	0.58	193	0.67	254	0.75	223	0.56	187
60	0.83	292	1.00	449	1.00	371	0.65	375
70	1.30	323	1.48	619	1.70	613	1.00	497
80	2.87	413	3.17	712	4.44	750	2.17	1143
85	4.22	520	4.52	832	6.83	1165	2.91	1458
90	8.65	748	9.17	1034	17.8	1543	5.91	1987

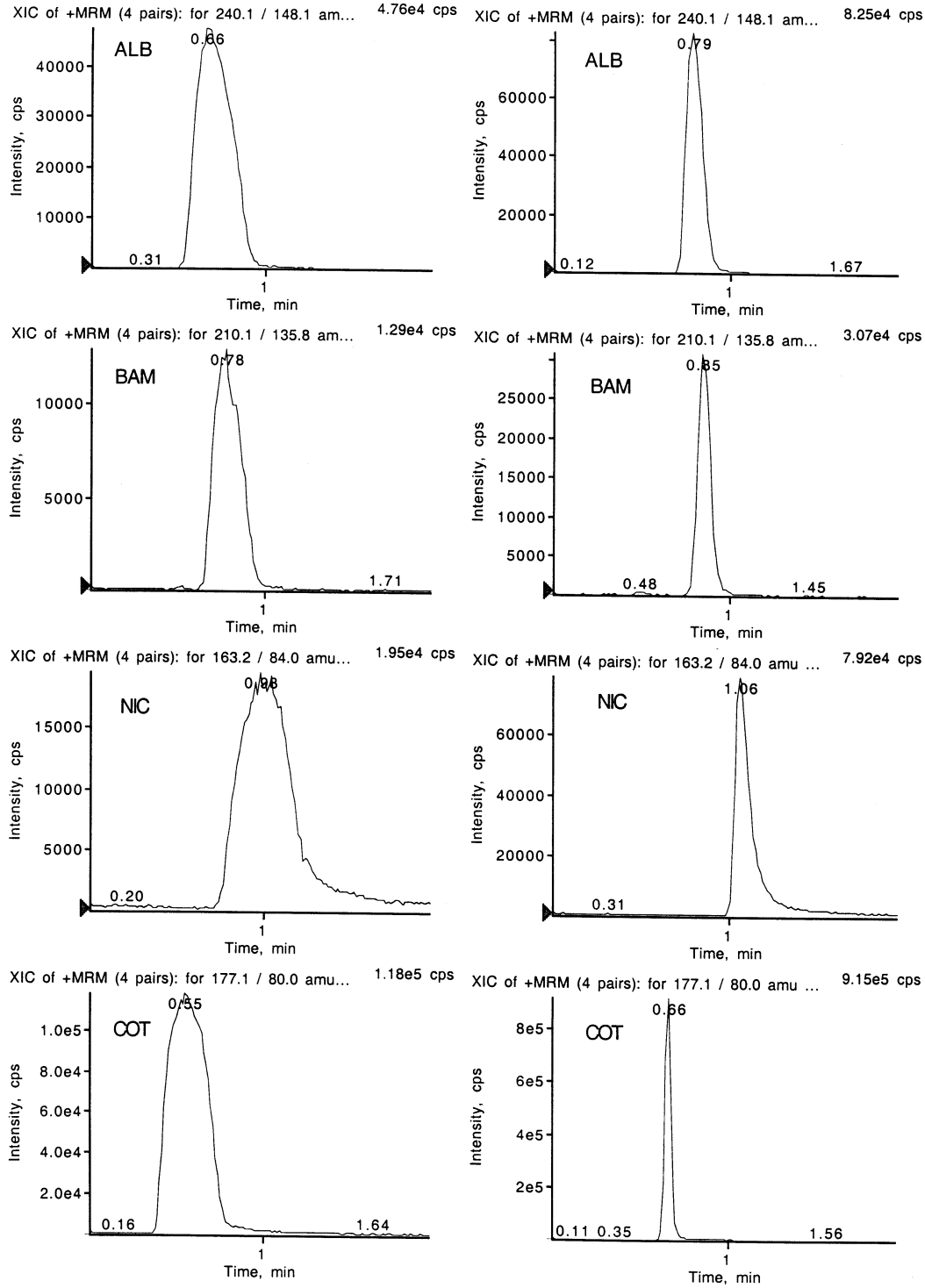

Fig. 2. *A mistake – assume that C_{18} column has reversed-phase retention mechanism*: Column: Hypersil BDS C_{18}, 50×2 mm^2 ID, 5 μm; mobile phase:water–acetonitrile–formic acid (20:80:0.2, v/v/v); flow rate:0.5 ml min^{-1}; injection solution: left panel: water; right panel: mobile phase ; injection volume: 10 μl.

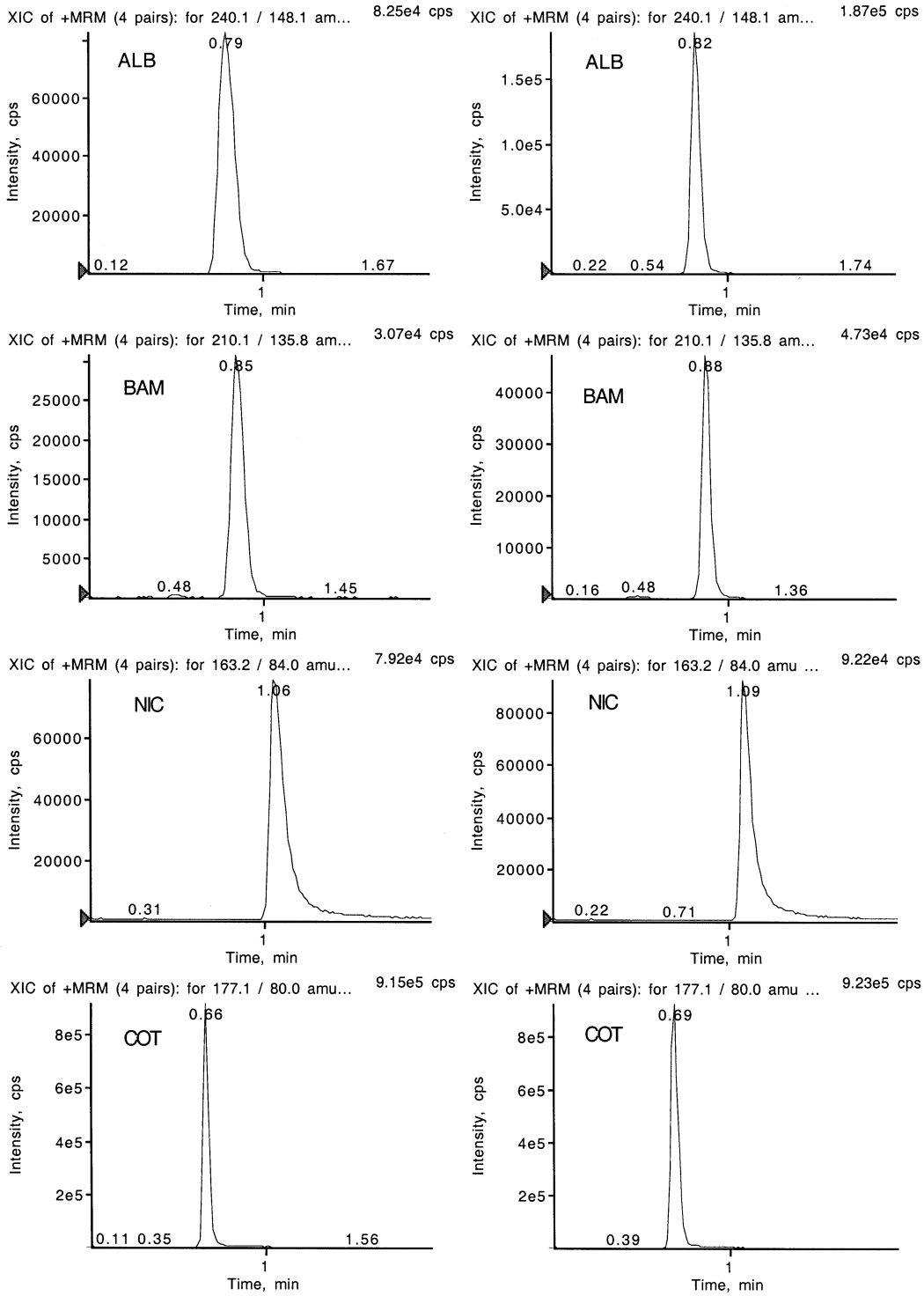

Fig. 3. *Improvement of chromatographic efficiency on Hypersil BDS C_{18} column*: Column: Hypersil BDS C_{18}, 50×2 mm^2 ID, 5 μm; mobile phase: water–acetonitrile–formic acid (20:80:0.2, v/v/v); flow rate:0.5 ml min^{-1}; injection solution: left panel: mobile phase; right panel: acetonitrile; injection volume: 10 μl.

Table 2
Influence of injection solution composition on capacity factor (k') and plate count (N)

Injection solution	ALB		BAM		NIC		COT	
	k'	N	k'	N	k'	N	k'	N
Water	1.87	38	2.39	99	3.26	45	1.39	22
Mobile phase	2.43	413	2.70	712	3.61	750	1.87	1143
Acetonitrile	2.56	1285	2.83	1418	3.74	1316	2.00	1231

tion mechanisms exist significantly (20–60% acetonitrile in mobile phase), the column efficiency is poorer. If the retention mechanism on a C_{18} column were blindly assumed to be reversed-phase, one would choose water as the weaker elution solvent and therefore the injection solution. Fig. 2 compares the chromatograms of all four compounds by using either water (left panels) or mobile phase (right panels) as the injection solution. Hypersil BDS C_{18} column and a mobile phase of acetonitrile–water–formic acid (80:20:0.2, v/v/v) were used. When water, the stronger elution solvent in this case, was used as the injection solvent, very poor peak shapes were observed for all compounds. For reversed-phase LC–MS–MS methods, which used mobile phases containing much higher content of organic solvents such as acetonitrile and methanol, mobile phases were often used as the injection solution [4–8]. This is a relatively safe approach if the retention mechanism is not determined. However, one has to be aware of the potential mismatch between the injection solution and mobile phase. In our laboratory, poor peak shape was observed when the samples were re-injected onto the LC–MS–MS system 24 h after the initial injections. The organic solvent, in this case acetonitrile, had a much higher evaporation rate than the water and the remaining portion had therefore more water than the mobile phase. This problem was overcome by re-diluting the sample with acetonitrile.

Even better chromatographic efficiency was obtained by using acetonitrile rather than mobile phase as the injection solution. The results were shown in Fig. 3. The capacity factors (k') and plate counts (N) of using water, mobile phase or

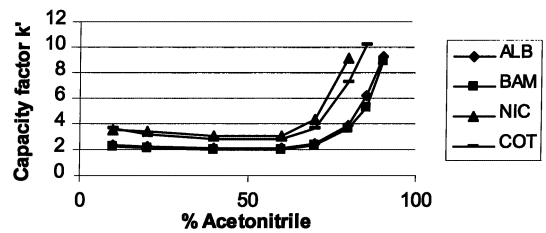

Fig. 4. *Influence of acetonitrile concentration in mobile phase on k' of basic compounds*: Column: Hypersil silica, 50 × 2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water–formic acid [x:(100 − x):0.2, v/v/v], where x varied from 10 to 90; flow rate: 0.5 ml min^{-1}; injection solvent: corresponding mobile phase; injection volume: 2 μl.

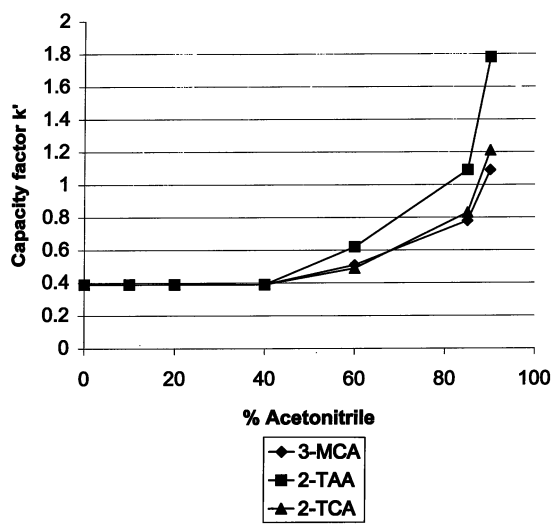

Fig. 5. *Influence of acetonitrile concentration in mobile phase on k' of acidic compounds*: Column: Betasil silica, 50 × 2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water [x:(100 − x), v/v], containing 5 mM ammonium acetate, where x varied from 0 to 92.5; flow rate: 0.5 ml min^{-1}; injection solvent: corresponding mobile phase; injection volume: 2 μl.

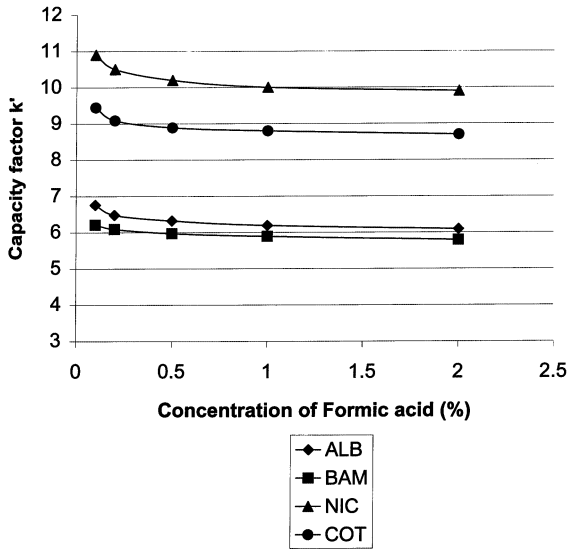

Fig. 6. *Influence of formic acid concentration in mobile phase on k′ of basic compounds*: Column: Hypersil silica, 50 × 2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water–formic acid [80:2:*x*, v/v/v], where *x* varied from 0.1 to 2; flow rate: 0.5 ml min^{-1}; injection solvent:corresponding mobile phase; injection volume:2 μl.

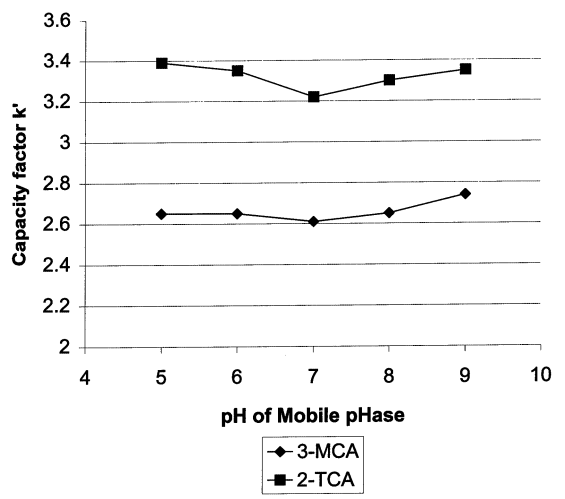

Fig. 7. *Influence of mobile phase pH on k′ of acidic compounds: column*: Inertsil silica, 50 × 2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water [92.5:7.5, v/v], containing 5 mM ammonium acetate. The pH of 5 mM ammonium acetate is adjusted with either acetic acid or ammonium acetate between 5 and 9; flow rate: 0.5 ml min^{-1}; injection solvent:corresponding mobile phase; injection volume: 2 μl.

acetonitrile as injection solvent were summarized in Table 2. When acetonitrile instead of mobile phase was used as injection solvent, the plate count increased three times for ALB, twice for BAM and NIC, and only slightly (10%) for COT. The sensitivity increase corresponded well with the increase of column efficiency. For ALB, more than 100% increase on sensitivity was observed.

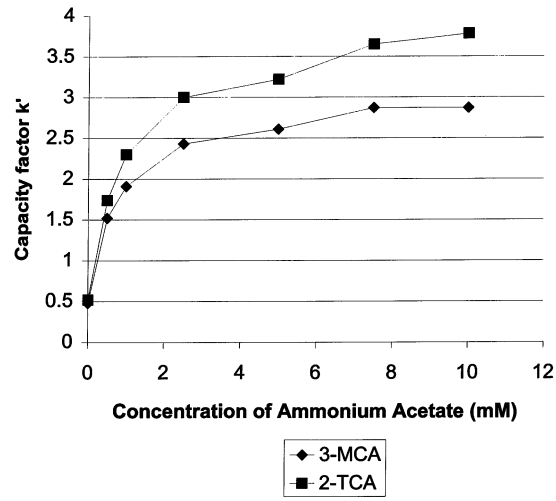

Fig. 8. *Influence of ammonium acetate concentration in mobile phase on k′ of acidic compounds*: Column: Inertsil silica, 50 × 2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water [92.5:7.5, v/v], containing *x* mM ammonium acetate, where *x* varies between 0 and 10; flow rate: 0.5 ml min^{-1}; injection solvent:corresponding mobile phase; injection volume: 2 μl.

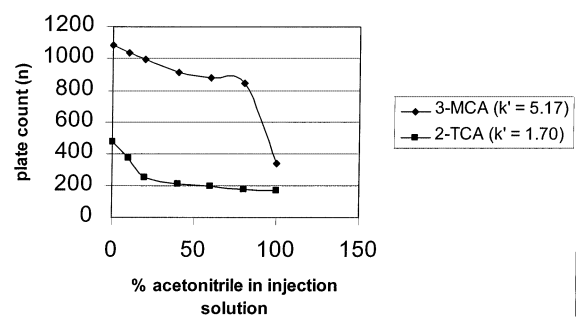

Fig. 9. *Influence of injection solution composition chromatographic efficiency for acidic compounds under reversed-phase retention mechanism*: Column: Hypersil BDS C_{18}, 50 × 2 mm^2 ID, 5 μm; mobile phase:water–acetonitrile (95:5, v/v) with 5 mM ammonium acetate; flow rate:0.5 ml min^{-1}; injection solvent:as indicated; injection volume:2 μl.

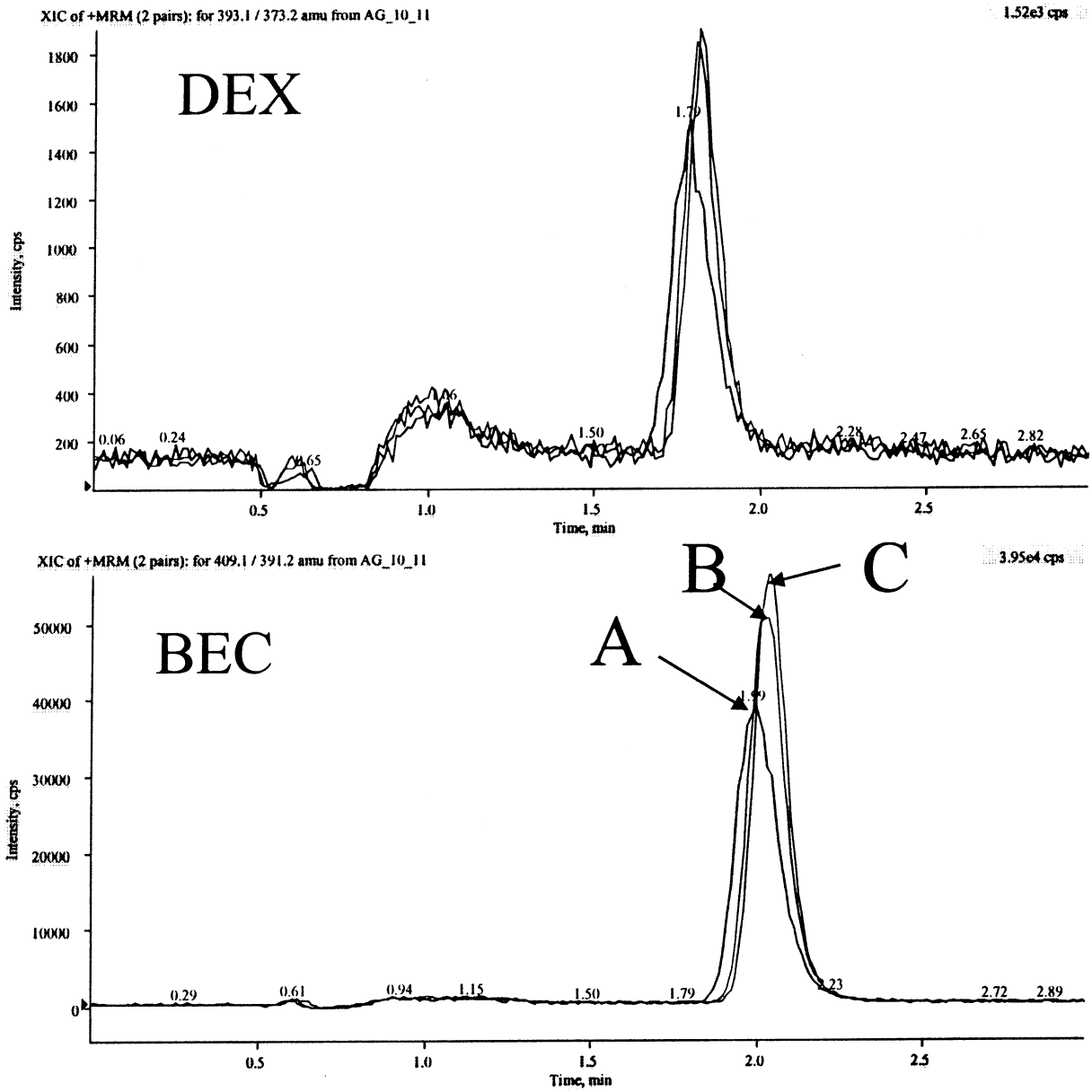

Fig. 10. *Influence of injection solution composition chromatographic efficiency for neutral compounds under reversed-phase retention mechanism*: Column: Hypersil BDS C_{18}, 50 × 3 mm^2 ID, 5 μm; mobile phase: water–acetonitrile–formic acid (65:35:0.1, v/v/v); flow rate:0.5 ml min^{-1}; injection solution: A: mobile phase; B: water–acetonitrile–formic acid (70:30:0.1, v/v/v); C: water–acetonitrile–formic acid (75:25:0.1, v/v/v); injection volume: 25 μl.

The capacity factors also increased upon using acetonitrile instead of mobile phase, indicating an on-column stacking effect. Although the reversed-phase C_{18} columns of another brand have similar mobile phase – retention profile similar to what shown in Fig. 1, the deflection point (the lowest retention) for each compound can vary significantly. Only minor retention time changes were

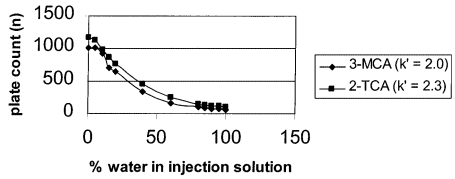

Fig. 11. *Influence of injection solution composition on chromatographic efficiency for acidic compounds under normal phase retention mechanism*: Column: Hypersil Si, 50 × 2 mm^2 ID, 5 μm; mobile phase:water–acetonitrile (7.5:92.5, v/v) with 5 mM ammonium acetate; flow rate:0.5 ml min^{-1}; injection solvent:as indicated; injection volume: 2 μl.

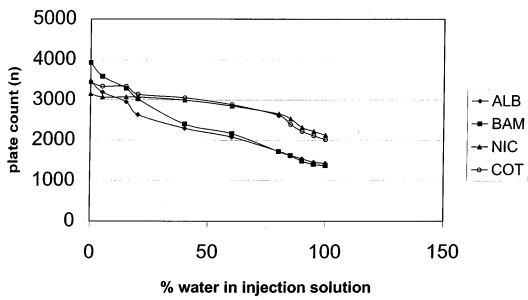

Fig. 12. *Influence of injection solution composition on chromatographic efficiency for basic compounds under normal phase retention mechanism*: Column: Hypersil Si, 50 × 2 mm^2 ID, 5 μm; mobile phase: water–acetonitrile–formic acid (30:70:0.2, v/v/v); flow rate: 0.5 ml min^{-1}; injection solvent:as indicated; injection volume: 2 μl.

observed when these columns were used under true reversed-phase conditions. However, the same brand columns from the different batches can have significant retention differences when used under normal conditions. This is probably due to the lack of consistent end-capping procedure, which leads to various amounts of the residual silanol groups left on the column. Because of this inconsistency, we did not recommend to use reversed-phase C_{18} columns under normal phase conditions. Nevertheless, the use of mobile phases containing highly organic solvents did provide significant sensitivity increases, probably due to the favorable spray conditions [16]. Therefore, we decided to pursue the investigation for the feasibility of using silica columns for normal phase.

On silica column with aqueous/organic mobile phases, the retention mechanism is relatively straightforward. Water is the stronger elution solvent. The results for basic and acidic compounds are shown in Figs. 4 and 5, respectively. The simplicity of the retention mechanism is important for rapid LC–MS–MS method development. The weaker eluting solvent is always the organic solvent. Fig. 6 shows the influence of formic acid concentration in the mobile phase on k' values of the basic compounds. Only slight decrease of analyte retention was observed upon increasing the acid concentration. For acidic compounds, mobile phase pH (when kept between 5 and 9) has minimal influence on the analyte retention (Fig. 7) while the ammonium acetate concentration in the mobile phase has more significant effect on the retention (Fig. 8). Retention time of the acidic analytes increased with the use of higher buffer concentration. The required sensitivity is more easily obtainable by using mobile phases of highly organic solvent content, because of favorable spray condition at the LC–MS–MS interface [16]. The silica columns provided reproducible column-to-column and batch-to-batch performance when used with aqueous-organic mobile phase.

3.2. Injection solution composition

3.2.1. Reverse-phase LC–MS–MS

Once the chromatographic retention mechanism is known, it is fairly simple to select the injection solution. We investigated the influence of the injection solution composition on the chromatography efficiency, defined as plate count (N). Fig. 9 shows the influence of injection solution composition on the acidic compounds, under reversed-phase retention mechanism. As expected, increasing acetonitrile in the injection solution decreased chromatography efficiency, particularly for the early eluting compound. Fig. 10 shows the chromatograms of the two neutral compounds, under reversed-phase retention mechanism. Again, better chromatography efficiency was obtained with a solution of elution strength weaker than that of the mobile phase.

3.2.2. Normal phase LC–MS–MS

Figs. 11 and 12 show the influence of water in injection solution on silica column efficiencies for acidic and basic compounds, respectively, under

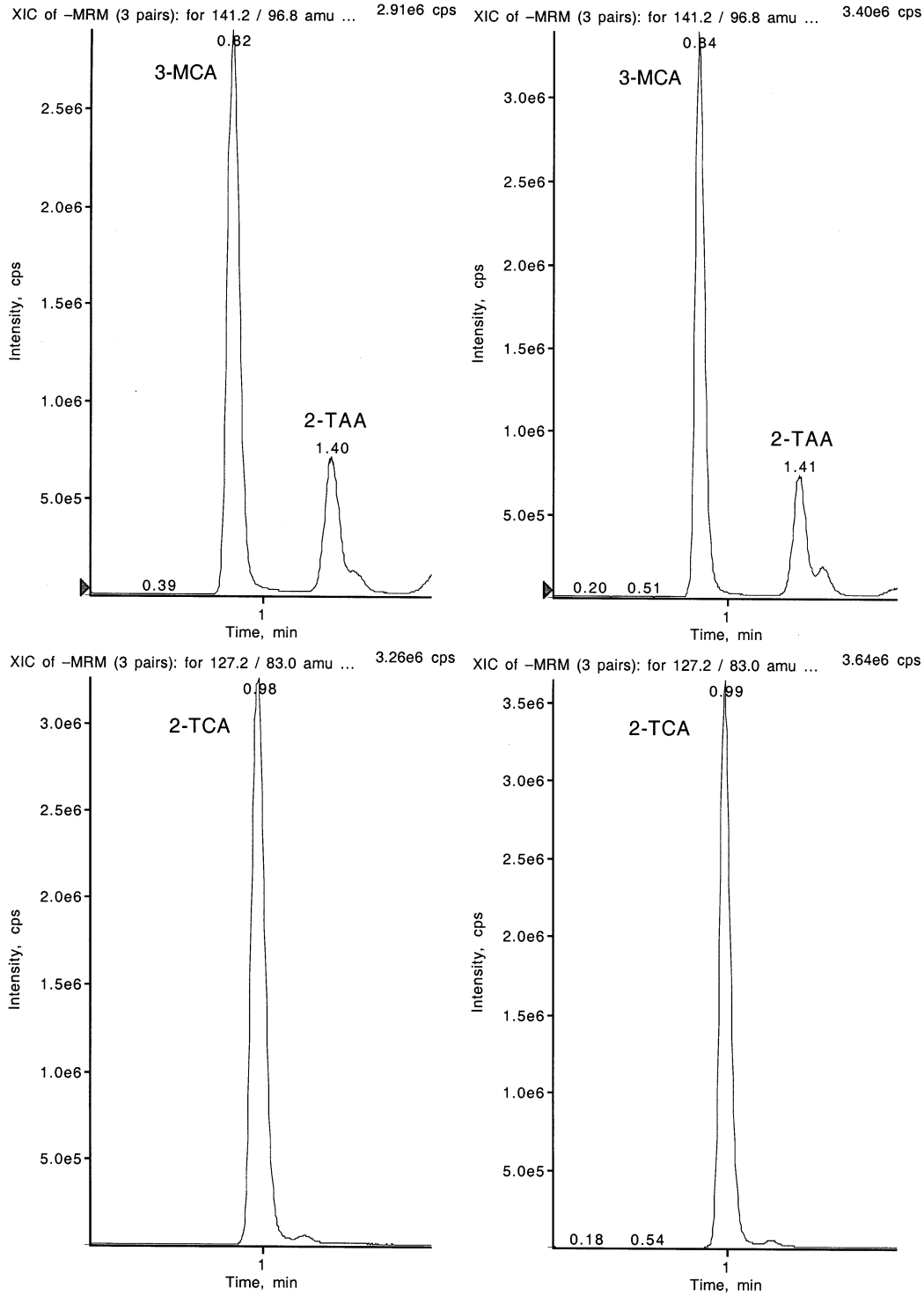

Fig. 13. *Chromatograms of LC–MS–MS of acidic compounds by using different injection solutions*: Column: Inertsil Si, 50×2 mm^2 ID, 5 μm: mobile phase: acetonitrile–water (92.5:7.5, v/v) with 5 mM ammonium acetate; flow rate: 0.5 ml min^{-1}; injection solution: left panel: mobile phase; right panel: acetonitrile; injection volume: 2 μl.

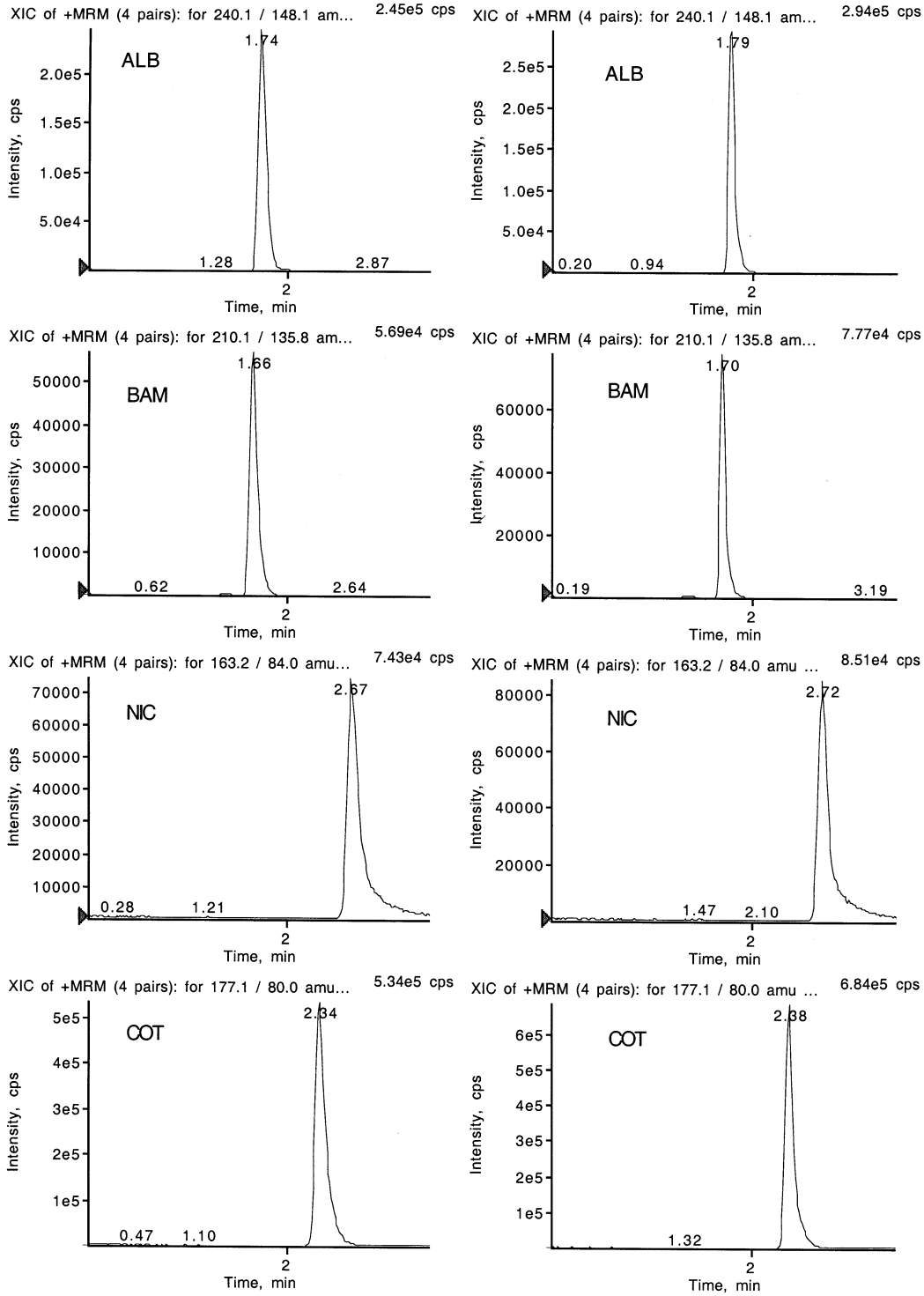

Fig. 14. *Chromatograms of LC–MS–MS of basic compounds by using different injection solutions*: Column: Hypersil Si, 50 × 2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water–formic acid (80:20:0.2, v/v/v); flow rate:0.5 ml min^{-1}; injection solution: left panel: mobile phase; right panel: acetonitrile; injection volume: 2 μl.

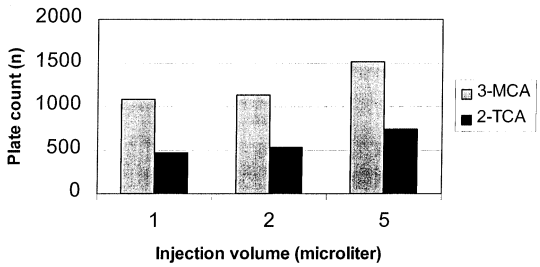

Fig. 15. *Influence of injection volume on reversed-phase chromatography efficiency*: Column: Hypersil BDS C_{18}, 50×2 mm^2 ID, 5 μm; mobile phase: water–acetonitrile (95:5, v/v) with 5 mM ammonium acetate; flow rate: 0.5 ml min^{-1}; injection solution: water

normal phase retention mechanism. Increasing the water content in the injection solution decreased chromatography efficiency. For basic compounds, the formic acid concentration (studied at 0%, 0.1%, 0.2%, 0.5%, 1%, and 2%) in the injection solution has no effects to the peak shape. The plate counts (N) for NIC, COT, ALB, and BAM remained to be approximately 3000, 3300, 3300, 4000, respectively. This was not a surprise since the analyte retention is virtually unaffected by the acid concentration in the mobile phase as shown in Fig. 6. For acidic compounds, neither the pH (studied at 5, 6, 7, 8, and 9) nor ammonium acetate concentration (studied at 0, 0.5, 1, 2.5, 5, 7.5, 10 mM) in the injection solution had effects on the peak shape. The plate counts (N) for 3-MCA and 2-TCA remained to be approximately 1200 and 1300, respectively. From Fig. 8, one would expect a

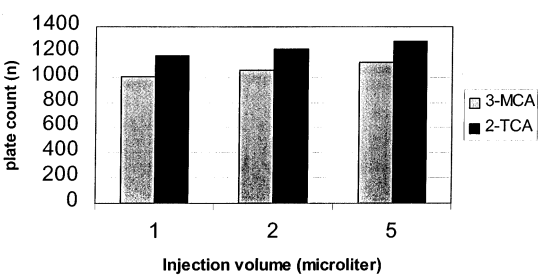

Fig. 16. *Influence of injection volume on normal-phase chromatography efficiency: Column*: Hypersil Si, 50×2 mm^2 ID, 5 μm; mobile phase: acetonitrile–water (92.5:7.5, v/v) with 5 mM ammonium acetate; flow rate: 0.5 ml min^{-1}; injection solution: acetonitrile.

decrease of the plate count by injecting analytes in a solution without buffer. However, this was not observed, probably due to the fact that only very small amounts of buffer (e.g. 0.5 mM) will increase the retention significantly. This small amount of buffer could be introduced into the injection zone by the diffusion of the mobile phase as discussed in Section 3.3. Normal phase LC–MS–MS methods for analysis of polar ionic analytes in biological fluids by using bare silica columns and aqueous-organic mobile phases have been reported [10,11,13,17,18]. Figs. 13 and 14 show that better chromatographic efficiency was once again achieved by using a solvent with weaker elution strength than mobile phase.

The analytes solubility in the injection solution deserves some comments. Acetonitrile is an excellent solvent for dissolving many polar and non-polar compounds. For example, the solubility of 2-TCA and 3-MCA is > 10 mg ml^{-1} in acetonitrile. From a practical bioanalytical point of view, unionized forms of the analytes are usually obtained either by solid phase extraction and liquid/liquid extraction procedures. These unionized analytes are usually soluble in acetonitrile, especially with the concentration range below μg ml^{-1}. Acetonitrile as the reconstitution and injection solvent has been successfully used [10,11,13]. Since most of the inorganic salts are poorly soluble in acetonitrile, use of acetonitrile as reconstitution and injection solvent has practical advantage for some of bioanalytical application. In our laboratory, we used acetonitrile to reconstitute five protease inhibitors after protein precipitation with acetonitrile and dry-down (unpublished results). Since salts were not dissolved in acetonitrile, we obtained very clean samples without matrix suppression. The detrimental matrix suppression for quantitative bioanalytical LC–MS–MS applications has been reported [19]. Small amounts of water and acid in the reconstitution and injection solvent has also been used for a few studies [17,18]. In these cases, the addition of water and acid is to prevent analytes from being adsorbed onto the glass surface. Usually, the amount of water in the injection solvent was still kept lower than the mobile phase to achieve on-column stacking effect.

The analyte stability in the injection solvent should also be considered. Compounds that are

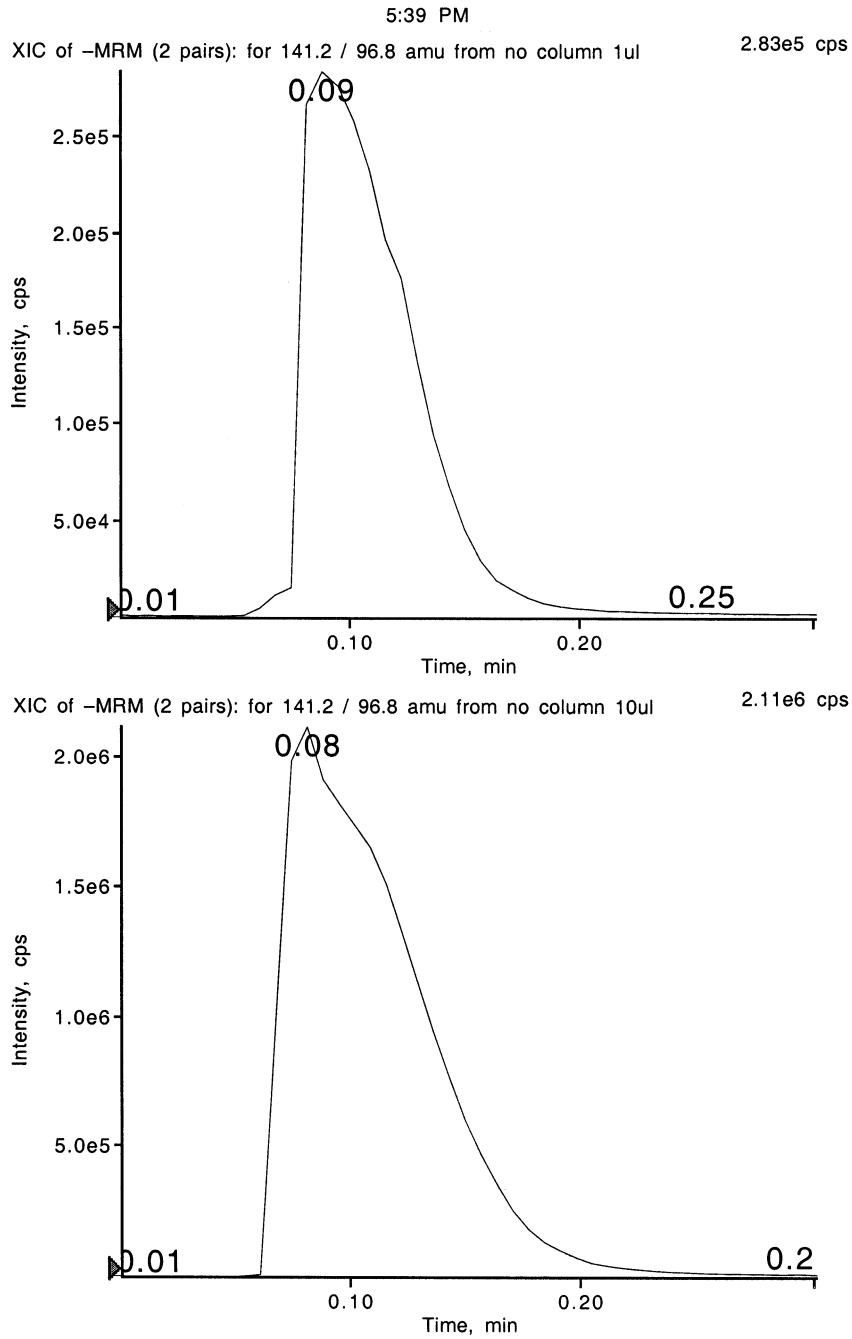

Fig. 17. *Flow injection chromatograms of 3-MCA*: mobile phase: acetonitrile–water (92.5:7.5, v/v) with 5 mM ammonium acetate; flow rate: 0.5 ml min^{-1}; injection solution: acetonitrile; injection volume: (upper panel) 1μl; (bottom panel) 10 μl.

unstable in the mobile phase may be stable in a weak elution solvent. For example, omeprazole is unstable in an acidic solution [20]. However, omeprazole is stable in acetonitrile and we have successfully used acetonitrile as the reconstitution solvent for LC–MS–MS analysis of omeprazole in human plasma (unpublished results). An acidic mobile phase was used to achieve maximum MS sensitivity. No on-column degradation of omeprazole was observed due to a relatively short analyte retention time (2 min) In this case, we achieved both analyte stability and on-column stacking by choosing acetonitrile as the injection solvent.

3.3. Injection volume

It was observed that injection volume could also influence the efficiency of chromatography. As expected, with an injection solution of elution strength equal to or stronger than the mobile phase, loss of chromatography efficiency was observed when the injection volume was increased. However, it was also noted that with an injection solution of eluting strength weaker than mobile phase, increased chromatography efficiency was observed upon increasing injection volume (Figs. 15 and 16). This could not be explained by the analyte stacking theory described in literature [3]. The stacking theory assumes that the plug of injected sample remains intact when it travels between the injector and the column. Since solvent of very weak elution strength is used as the injection solvent, the analytes will then be focused at the top of the analytical column as a very narrow band, independent of injection volume. The elution of the analytes in the axis of the column is not realized until the mobile phase contacts the analytes. However, what actually happened after the injection was a constant diffusion of injection plug zone and the mobile phase zone, resulting in a Gaussin (unsymmetrical) peak instead of a rectangular zone as would be observed if the injection plug remained untouched. Fig. 17 shows flow injection chromatograms of 3-MCA without a column. A very quick arising at the front part of the peak indicates minimal diffusion of the injection zone front and the mobile phase. The tail of the peak indicates that more diffusion occurs at the end of the injection zone. In other words, a gradient elution was created at the end of injection zone. A larger injection volume leads to a longer tail of the peak, therefore a longer gradient elution in the column and a better peak efficiency. More study is needed to fully understand the phenomenon of the gradient elution created by injection solvent.

4. Conclusion

Selection of appropriate injection solution should be carefully evaluated, particularly for fast LC–MS–MS methods. The retention mechanism should be determined and C_{18} column should not be assumed to be reversed-phase. Peaks eluting close to solvent front (poor retention) are more easily disturbed by the injection solution mismatch. Better peak shape and higher chromatographic efficiency can be obtained by using the weaker component in the mobile phase as the injection solution.

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