Methylammonium Formate as a Mobile Phase Modifier For Totally Aqueous Reversed-Phase Liquid Chromatography

ARTICLE *in* JOURNAL OF CHROMATOGRAPHY A · OCTOBER 2008

Impact Factor: 4.17 · DOI: 10.1016/j.chroma.2008.09.064 · Source: PubMed

2 AUTHORS, INCLUDING:

Neil D Danielson
Miami University
144 PUBLICATIONS **2,216** CITATIONS

SEE PROFILE

Published in final edited form as:
J Chromatogr A. 2009 April 17; 1216(16): 3578–3586. doi:10.1016/j.chroma.2008.09.064.

Methylammonium formate as a mobile phase modifier for reversed-phase liquid chromatography

Shau Grossman and **Neil D. Danielson***
Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA

Abstract

Although alkylammonium ionic liquids such as ethylammonium nitrate and ethylammonium formate have been used as mobile phase "solvents" for liquid chromatography (LC), we have shown that methylammonium formate (MAF), in part because of its lower viscosity, can be an effective replacement for methanol (MeOH) in reversed-phase LC. Plots of log retention factor versus the fraction of MeOH and MAF in the mobile phase indicate quite comparable solvent strength slope values of 2.50 and 2.05, respectively. Using a polar endcapped C18 column, furazolidone and nitrofurantoin using 20% MAF-80% water could be separated in 22 min but no baseline separation was possible using MeOH as the modifier, even down to 10%. Suppression of silanol peak broadening effects by MAF is important permitting a baseline separation of pyridoxine, thiamine, and nicotinamide using 5% MAF-95% water at 0.7 mL/min. Using 5% MeOH-95% water, severe peak broadening for thiamine is evident. The compatibility of MAF as a mobile phase modifer for LC with mass spectrometry detection of water soluble vitamins is also shown.

Keywords

Ionic liquid; methyl ammonium formate; mobile phase; liquid chromatography

1. INTRODUCTION

Room temperature ionic liquids (ILs), usually composed of an organic cation and an inorganic or organic anion, are non-volatile, quite viscous, and considered environmentally friendly to the atmosphere as compared to conventional organic solvents. The primary uses of ILs in separation science include liquid-liquid extraction, solid phase microextraction, stationary phases for GC and LC, and mobile phase additives for LC and CE. Several recent reviews summarizing the application of ILs to separation science have been written [1–4]. Most ILs such as the imidazolium or pyridinium classes are expensive in bulk quantities, have high viscosities, and have a high UV wavelength absorbances. Therefore their use as mobile phase modifiers for liquid chromatography (LC) has been limited to the millimolar (mM) level. Catecholamines such as norepinephrine, epinephrine, and dopamine could be separated faster with less peak tailing on a C18 column using 25 mM 1-butyl-3-methylimidazolium chloride in an aqueous mobile phase [5]. Control of solute retention using alkyl-methyl-imidazolium ILs at the mM level through ion-pairing, C18 stationary phase adsorption, and masking of silanols was delineated [6]. Alkylammonium nitrates have been used as organic solvent

E-mail address: E-mail: danielnd@muohio.edu (N. D. Danielson).

replacements for the mobile phase in reversed-phase LC [7,8] However these compounds show strong UV absorbance in the 260–320 nm range [7] and are not compatible with fluorescence detection due to the electron withdrawing character of nitrate [9]. Chromatographic applications were very limited, primarily to nitro aromatics [8].

Previously, a new class of ionic liquids, alkylammonium formates (AAFs) such as ethylammonium formate (EAF), n-propylammonium formate (PAF), and n-butylammonium formate (BAF) have been synthesized and investigated as mobile phase alternatives to organic solvents for reversed-phase LC with spectrophotometric detection [10]. Kamlet-Taft solvatochromic measurements for AAFs have shown that their hydrogen-bond acidities and basicities are similar to those for methanol but their polarizabilities are like acetonitrile. The low UV background absorbance of about 250 nm and a relatively low viscosity of about 11.5 cP have made EAF a particularly desirable organic solvent alternative for reversed-phase LC as compared to PAF and BAF. A comparison of EAF with methanol (MeOH) as a mobile phase "solvent" for reversed-phase LC has also been made [11].

Recently, it has been reported by Greaves et al. [12] that methylammonium formate (MAF) is also a room temperature IL. In this work, the authors investigated the physicochemical properties and phase transitions of 25 protic ionic liquids including MAF and EAF. MAF was reported to have the highest conductivity and the lowest viscosity followed by EAF. However, no details on how these ILs were synthesized were given and the solvent properties for separation science were not studied.

In this work, after a controlled synthesis, MAF was characterized with respect to polarity by spectroscopy, and then as an organic solvent replacement for reversed-phase LC with UV detection. Resolution of nitrofuran drugs was improved using MAF as compared to MeOH. To the best of our knowledge, the potential of an IL as a major mobile phase component compatible with LC-MS was demonstrated for the first time using MAF. The long-term goal of this research was to show that this characterization of MAF as a mobile phase component for reversed-phase LC of small molecules should facilitate the future application of MAF as a mobile phase modifier for the separation of proteins.

2. EXPERIMENTAL

2.1 Instrumentation

For spectroscopic measurements, fluorescence spectra were recorded on Perkin-Elmer Model LS50B and LS55 luminescence spectrometers with a 1×1×4 cm quartz fluorescence cell. UV-Vis absorbance spectra were measured using either an Agilent 8453 UV-visible spectrophotometer with a 1×1×4 cm quartz cell and or a Hewlett-Packard 8452A diode array spectrophotometer. A Perkin-Elmer Spectrum One FT-IR spectrometer with a Universal ATR Sampling Accessory was used to measure IR spectra. ^{1}H NMR and ^{13}C NMR spectra were recorded on either a Bruker 300 or 500 MHz NMR spectrometer.

For viscosity measurements, the Cannon-Fenske 100/N204 Routine Viscometer (for transparent liquids) equipped with a Cannon CT-1000 constant temperature mineral or silicon oil bath was used (Cannon Instrument Co., State College, PA, USA). Triplicate measurements were taken for each temperature. The time average was used to compute the viscosity $\eta = t_{AVG} \times const \times density$ where *const* is the temperature constant (0.01438 for 24,40,60,80°*C* and 0.01431 for 100°*C*) provided by the Certificate of Calibration for Viscometer No. 100 N204 (Cannon Instrument Co.).

Conductivity measurements were conducted using Oakton Con 6 meter obtained from Cole-Parmer (Vernon Hills, IL, USA).

For HPLC experiments, the separations were done using either a Varian Polaris C18-A 3 μm (50 × 4.6 mm) column, a Phenomenex Aqua C18 5 μm 200 Å (150 × 4.6 mm) column, a Synergi 4 μm MAX RP-80A (150 × 4.6 mm) C12 column, or a PRP-1 10 μm (150 × 4.1 mm) column. A SSI 222C HPLC pump, a model 7010 Rheodyne injector (20 μL loop), and a Shimadzu SPD-6AV UV-Vis spectrophotometric detector made up the HPLC instrument. Data acquisition was done using the SRI EasyChrom computer software. Effective band retention time, $t'_R = t_R - t_0$, and band width at half-height, $W_{½}$, were taken from triplicate measurement at each flow rate ranging from 0.2 mL/min to 2.0 mL/min to compute the plate count using $N = 5.54\left(\frac{t'_R}{W_{1/2}}\right)^2$. Height equivalent of a theoretical plate (HETP) values were calculated using $H = \frac{L}{N}$.

For GC experiments, a Shimadzu GC-17A gas chromatograph using a flame ionization detector was used. The Restek RTX-1701 (14% cyanopropylphenyl) - methylpolysiloxane, bonded and crosslinked at 1.0 μm film thickness stationary phase) capillary column with dimensions of 30 m × 0.53 mm I.D. was operated at 35 °C. The internal standard method using n-propanol was used to compensate for the variation in the 0.2 μL injection size for the determination of methanol.

The Perkin-Elmer thermogravimetric analyzer TGA7 and thermal analysis controller TAC 7/DX were used to collect thermogravimetric analysis (TGA) traces.

The vacuum freeze-drying instrument is composed of a motor from General Electric, the vacuum pump from Precision Scientific Co., Chicago, IL, USA, the belt from GCA Precision Scientific, and the ice trap from Virtis Co., Gardiner, NY, USA.

For the Karl Fischer titration experiment, a Metrohm 751 GPD Titrino instrument was used; 100 μL samples were weighed by difference on a analytical balance in a glove box and then capped before transfer for titration under N_2 purge.

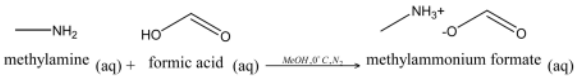
For LC-MS, the LC instrument is Hewlett-Packard Series 1100 (with auto sampler) with ChemStation (Instrument 1) software and the MS instrument is Bruker with ESQUIRE-LC software. For LC/MS using electrospray ionization (ESI) with positive ion polarity, the following parameters were set: trap drive 30.7, capillary exit 103.3 v, nebulizer 60 psi, dry gas 8 L/min for 0.2 mL/min LC flow rate, dry temperature 365 °C, HV capillary 4500 V, HV end plate offset -500V, spectral averages 8. For LC/MS using atmospheric pressure chemical ionization (APCI) with positive ion polarity, the following parameters were changed: trap drive 29.6, capillary exit 90.8 v, nebulizer 65 psi, dry gas 4 L/min for 0.7 mL/min LC flow rate, dry temperature 350 °C, APCI temperature 450 °C, corona parameter +3000 nA.

2.2 Chemical reagents

Methylamine (CH_3NH_2, 33% w/w in absolute ethanol solution, MW=31.06 g/mol, density=0.756 g/mL) was purchased from Aldrich (Milwaukee, WI, USA). The formic acid (HCO_2H, 98% assay, MW=46.03 g/mol, density=1.22 g/mL) was purchased from Fluka (St. Gallen, Switzerland). Methanol, acetonitrile were HPLC-grade produced by Pharmco Products (Brookfield, CT, USA). Organic solutes, caffeine anhydrous (FW=194.19, from Ward's Natural Science Establishment, Rochester, NY, USA), p-nitroaniline ($p\text{-}O_2NC_6H_4NH_2$, FW=138.13, from Matheson Coleman & Bell, Norwood, OH, USA), and phenol (C6H5OH, FW=94.11, from Fisher Scientific, Fair Lawn, NJ, USA), were of analytical grade. Organic solutes, 2-nitroaniline (98%), 4-nitrophenol (99+%), trans-4-methoxy-β-nitrostyrene (99%), and 4-nitroanisole (97%) were obtained from Aldrich. 4,4′-Bis(diethylamino)benzophenone

(> 98%) was obtained from Fluka. Reichardt's dye (2,6-diphenyl-(2,4,6-triphenyl-N-pyridino) phenolate) was purchased from Sigma-Aldrich (St. Louis, MO, USA). N,N-Dimethyl-4-nitroaniline (CAS 100-23-2) was obtained from Lancaster Synthesis, Windham, NH, USA. Activated carbon Darco G-60-100 mesh powder used for solid-phase extraction (SPE) was purchased from Sigma-Aldrich. Deuterated methanol (CD_3OD) was purchased from Aldrich. Distilled, deionized water was prepared using a Barnstead Millipore unit (Dubuque, IA, USA). The water-soluble vitamins ascorbic acid, nicotinic acid, thiamine hydrochloride, and niacinamide were obtained from Sigma-Aldrich. Karl Fischer reagent and matching solvent, two-component reagent Hydranal, were purchased from Riedel-de Haen.

2.3 Synthesis of methylammonium formate

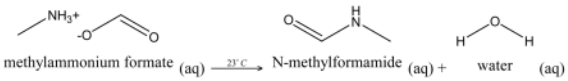


The reaction was carried out at0°*C*, which is much lower than 40°*C* from the previously reported synthetic procedure [12]. An equimolar amount of the formic acid (98%, from Fluka) solution diluted with 50:50 HPLC-grade MeOH was slowly added into the chilled methylamine (33% wt. solution in absolute ethanol, from Aldrich) at the drip rate of every 2~3 seconds per drop. Both methylamine and the formic acid solution were chilled before the reaction started. The use of an Aldrich addition funnel with PTFE needle valve controlled the drip rate of the formic acid and methanol solution. The addition funnel (with jacket) was connected to an icy water pump to keep the formic acid solution cold throughout the reaction. The chilled methylamine was kept in a three-neck round bottom flask, sitting in the bath of ice and dry ice mixture, capped with a high-vacuum valve adapter (to minimize the escape of methylamine) and a nitrogen gas inlet adapter (to dry the reaction flask). The reaction solution mixture is gently stirred throughout the reaction. The nitrogen gas was gently bubbled into the solution mixture for the first 15 to 20 minutes before it was left above the solution for the remaining reaction time. A vacuum pump was applied to remove the ethanol/methanol in the product. Vacuum freeze-drying of MAF was carried out for 48 hours to remove the water and residual alcohol content after vacuum solvent extraction. Residual methanol was found to be less than 0.05% by GC however MAF interfered with the ethanol peak and it could not be quantitated. For every 250 mL methylamine (33% wt. solution in absolute ethanol), 155 mL of MAF was synthesized after solvent extraction. Like other primary amine based AAFs (10), MAF was found to be a clear, colorless liquid. It was observed that at high reaction temperature (≫0° *C*) or if the starting materials are not fresh the reaction product appeared to be yellowish but clear. Spectroscopic characterization by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and UV-Vis spectroscopy was then carried out to determine the purity of synthesized MAF.

3. RESULTS AND DISCUSSION

3.1 Characterization of MAF

3.1.1 Spectroscopic analysis—The identification of MAF was carried out using IR and NMR spectroscopic analysis. The IR spectrum showed characteristics of C-H stretching (for NCH_3) at 2790 (in the range of 2850~2700) cm^{-1}, C-H bending (for CH_3) at 1467 (between 1470~1430) cm^{-1}, and 1351, 1379 (between 1370~1380) cm^{-1}, N-H stretching at 3329 (between 3500~3310) cm^{-1}, N-H bending at 1591 (1650~1550) cm^{-1}, C=O stretching at 1668 cm^{-1}, and C-O stretching at 1121 (1050~1150) cm^{-1}. The ^1H NMR spectrum of MAF in deuterated methanol CD_3OD shows characteristic chemical shifts for methyl proton at 2.5 and 2.7 ppm, formate proton at 8.5 ppm. In a MAF batch that was stored at room temperature for 5 weeks, a small formamide proton at 8.0 ppm was evident from the presence of N-methylformamide [13].



This slow degradation of MAF agrees with a previous report [12]. The ^{13}C NMR spectrum of MAF in deuterated methanol CD_3OD shows a characteristic chemical shift of formate carbon at 169 ppm, methyl carbon at 24 ppm. The amide carbon at 163 ppm is from the degraded product N-methylformamide.

The same MAF sample was left in the NMR tube and kept air-tight in the freezer (−9.5 °*C*) for subsequent measurements by ^{13}C NMR at 5, 14, and 28 weeks. The % amide carbon with respect to the formate peak values at 5 and 14 weeks were 5.5 and 5.7, respectively and that at 28 weeks was 5.0. The same NMR sample tube was then placed in the refrigerator (maintained at the temperature just above 0° C) and used to measure the % peak height of the amide carbon to the formate carbon at 1, 3, 5, and 6 weeks. The corresponding % amide carbon formation values were 13.25, 12.99, 13.25, and 12.8. The stability of MAF can be considered markedly improved when stored at a temperature near 0°*C*.

The UV-Vis spectrum of undiluted MAF indicated the UV-cutoff (absorbance units (AU) = 1) at 254 nm. However, the absorbance decreases rapidly above this wavelength to 0.15 AU at 260 nm and essentially to 0 at 270 nm. LC detection of aromatic compounds was always facile using MAF mobile phases. The fluorescence background as measured by emission at 414 nm of undiluted MAF can be reduced by 30% by solid phase extraction using 0.1 g of activated carbon Darco G-60-100 mesh powder for every 5 mL of MAF.

3.1.2 Physical and chemical properties of MAF

pH of MAF: The measured pH value of undiluted MAF is 7.4. It agrees with the amphiprotic calculation using the pKa values for methylamine (10.64) and formic acid (3.8).

Density: Triplicate measurements of 2 mL of SPE extracted MAF measured using a Gilson metered syringe were taken with the Mettler analytical balance. The average density of undiluted MAF is 1.05 g/mL. Using weight by difference in the glove box to minimize the exposure to the moisture in the air due to the highly hygroscopic nature of MAF, five measurements of 100 μL sample (without the SPE treatment) were taken after vacuum freeze-drying for 120 h and then left at room temperature for two weeks without desicator storage before the measurements. The average density is 1.06 g/mL, similar to a previously reported value at 1.087 g/mL [12].

Karl Fischer Titration: The water content of MAF was determined by a volumetric method using the Karl Fischer titration. Vacuum freeze drying was applied before the titration. Triplicate measurements of the water content in 100 μL of MAF were carried out. Due to the highly hygroscopic nature of MAF, samples were weighed and transferred in the oxygen-free glove box. The averaged water content for 48-h-freeze-dried MAF (stored in a desiccator that was placed in the refrigerator) was 1.5±0.1% without the introduction of nitrogen purge. Similar measurements were also carried out using nitrogen purge during the titration for the MAF sample freeze-dried for 120 hours and subsequently left at room temperature without desicator storage for two weeks before the experiment. The consequent water content is 1.7 ±0.2% (n=3). Although we followed a similar drying procedure, this is about four times higher than a previously reported water content of MAF [12].

Thermogravimetric analysis (TGA): The undiluted MAF sample was heated from 25°*C* up to 550°*C* and at the same time the TGA traces were collected at the scan rate of 10°*C*/min. The traces showed the weight loss starting gradually from 50°*C* to 150°*C*. After 150°*C*, the

degradation proceeded rapidly. At 288°*C* the sample was completely vaporized leading to a zero final mass. This is virtually the same as a previous reported profile [12].

Conductivity: Conductivity measurements of volume percent of MAF in water were taken at 25°C at 10% intervals. Triplicate measurements were taken for every data point. The average standard deviation across various volume percent MAF (in water) was 2.26 milliSiemans (mS). From 10–40% MAF in water, the conductivity in mS increased from 81 to 172. From 50–100% MAF, the conductivity values decreased from 170 to 65. The profile is similar to that previously published for EAF [10]. There is increasing ionization up to 40~50% MAF in water and the increasing viscosity and/or ion-pair aggregation beyond 50% MAF. The peak point at 40% MAF represents the critical aggregate concentration (CAC) of MAF. The CAC value for MAF is estimated to be about 4.6 M, which agrees with the previously reported CAC values for alkylammonium formates (AAFs) [10].

Viscosity: Viscosity measurements at room temperature (n=3) were carried out for overnight vacuum freeze-dried MAF and this average viscosity was 9.05 cP; the viscosity for the same freeze-dried MAF sample with 1% water added was 8.78 cP. The viscosity of MAF was also reduced approximately about 14% ~ 15% after six months' exposure to open air and moisture. The presence of small amounts of water definitely can decrease the viscosity of MAF. Possibly due to the use of a viscometer instead of rheometer, our viscosity is about a factor of 1.8 less than that reported previously [12]. However, the viscosity of MAF is about 20% less than that for EAF making it one of the least viscous IL known.

The plot of ln viscosity of freshly prepared MAF as a function of the inverse of temperature for 5 points from 25°*C*, 40°*C*, 60°*C*, 80°*C*, 100°*C* indicated a correlation coefficient better than 0.99 as shown in Figure 1. The inverse temperature dependence is in the form of Arrhenius equation $\ln\eta = \ln\eta_{\inf} + E_\eta/RT$ where η is the viscosity in *mPa* • *s* or *cP*, $\eta_{\inf}$ is the viscosity at the infinite temperature, E_η is the activation energy in *kJ/mol* for viscous flow, $R = 8.314 \times 10^{-3}\ kJ(mol^{-1}\ K^{-1})$, the gas constant, and T is the temperature in Kelvin. The linear regression analysis of parameters $\ln\eta$ with $1/RT$ gives E_η =14.5 *kJ/mol* for MAF. A similar plot of ln viscosity of MAF (exposed to open air for 6 months) as a function of the inverse of temperature gave only a slightly different value of E_η =15.5 *kJ/mol*. E_η is considered as a measure of the energy barrier required for the IL (ionic liquid) ions to move past each other, which could be due to the physical size or just a strong interaction of the ions. This value of E_η or MAF is significantly less than 26 kJ/mol reported previously for EAF [10].

The plot of ln viscosity as a function of the fraction of MAF in water at room temperature is also shown in Figure 1. As reported previously for EAF [10], the plot shows a slight upward curvature and can be fit to a third order polynomial $y = A_1 + A_2x + A_3x^2 + A_4x^3$ where y = ln viscosity and x = volume percent of MAF in water (Figure 1). The inverse ln of A1 of 0.94 as expected is quite similar to the viscosity of water (0.89 cP). Dilution with water is effective in reducing the viscosity of MAF over the entire range unlike water-MeOH or water-acetonitrile compositions which show a maximum viscosity near 50–60% of the organic solvent in water [14].

Polarity: The polarity of MAF was first measured by $E_T(30)$ and the normalized value E_T^N using the Reichardt's betaine dye [15]. $E_T(30)$ is defined as the molar electronic transition representing the blue shift of the UV-Vis absorption band of the zwitterionic betaine dye. The blue shift is caused by the differential solvation of the highly dipolar ground state and less dipolar first excited state of the dye; with increasing polarity, the ground state molecule is better stabilized by solvation than the molecule in the excited state. E_T^N is called the normalized value with a range from 0 for the least polar solvent tetramethylsilane (TMS) to 1.0 for water, the most polar solvent. For MAF after 48 hr freeze drying, the absorption peak occurs at 477

nm which calculates to respective $E_T(30)$ and E_T^N values of 59.9 and 0.9. A plot of the E_T^N values for various alkylammonium ILs [16], MeOH, acetonitrile, and water shows MAF has a similar polarity to MeOH and the other AAFs (Figure 2).

The polarity of MAF was also characterized by the Kamlet-Taft scale which is composed of three terms: π* (dipolarity/polarizability, α (hydrogen bond acidity), and β (hydrogen bond basicity) [17,18]. The longest wavelength band of the three probe molecule spectra (4-nitroanisole, N,N-dimethyl-4-nitroaniline, and trans-4-methoxy-β-nitrostyrene) taken in MAF were measured to determine the π* dipolarity/polarizability which best correlates solvatochromic effects for n -> π* and π-> π* electronic spectral transitions. For 48-h-freeze-dried MAF, the average π* value is 0.99 which was similar to the MAF sample without freeze drying. This dipolarity/polarizability value for MAF is similar to that of water (1.1) as compared to MeOH (0.6) and acetonitrile (0.75). The π* values for AAFs progress steadily downward as the alkyl chain increases in length from about 1 for MAF to 0.7 for BAF.

The longest wavelength of the UV-Vis absorption band in the spectra for the two probe molecules Reichardt's betaine dye and 4,4-bis(diethylamino)benzophenone taken in MAF were measured to determine the hydrogen bond acidity or proton donating capability (α). For 48-h-vacuum-freeze-dried MAF, the average a value is 0.91. This hydrogen bond acidity of MAF is more similar to that of MeOH (0.93) as compared to acetonitrile (0.2) and water (1.2). The other AAF ILs had similar α values ranging from 0.85 to 0.89 [10].

The hydrogen bond basicity or the capability as proton acceptor or electron pair donor of MAF (β value) was determined using the three probe molecules 2-nitroaniline, 4-nitroaniline, and 4-nitrophenol. For 48-h-vacuum-freeze-dried MAF, the average β value was 0.69. This β value for MAF is very similar to that of MeOH (0.62) and very dissimilar to that of MeCN (0.31) or water (0.18). The β value for the other AAFs increase steadily from that for MAF to 0.78 for BAF [10].

3.2 MAF as a major mobile phase component for LC

The column back pressure was profiled at 1 mL/min as a function of the volume percent of either MeOH or MAF in water as the mobile phase using the Polaris C18-A 3 μm column (50 × 4.6 mm). The column back pressure for both MeOH and MAF were comparable at about 40% or lower composition, ranging from 7.6 to 10.3 MPa (1.1–1.5 kpsi) for MeOH and 8.3 to 11.7 MPa (1.2 –1.7 kpsi) for MAF. MAF has a higher back pressure from 50–70% in mobile phase composition of 13.8–20 MPa (2–2.9 kpsi) due to its higher viscosity than that of MeOH. The higher back pressure for 50-50 MeOH-H_2O than either 100% MeOH or 100% water, giving a parabolic profile, is analogous to viscosity versus organic solvent-water composition plots [14]. In general, short wider diameter columns with moderate reversed-phase retention (C18 with polar endcapping) would be recommended for use with MAF mobile phases.

Average logarithm of retention factor log k′, where $\log k' = \log k_w - S\Phi$, of p-nitroaniline (n=3) as a function of the volume percent of MAF or MeOH in water was plotted, where Φ is the volume percent of MAF and *S* is a measure of organic solvent strength dependent on solute structure. The same Polaris C18 column was used. The linear regressions are $y = -0.0205x + 1.4939$ with the R^2 value of 0.98 for using MAF as volume percent in water from 20–70% (n=6) and $y = -0.0248x + 1.325$ with the R^2 value of 0.99 for using MeOH as volume percent in water from 20–70% (n=6). The solvent strength of 2.05 for MAF is somewhat less than that for MeOH (2.48) but certainly MAF could be considered as a practical modifier solvent for control of reversed-phase retention. Previously reported *S* values for EAF, PAF, BAF, and MeOH were 1.90, 2.20, 2.50, and 2.55, respectively, based on the plot of retention factor for p-nitroaniline as a function of volume percent of organic modifier (EAF, PAF, BAF, and MeOH) using a PRP-1 column [10].

3.2.1 Height equivalent of a theoretical plate (HETP) plots—Van Deemter plots using mobile phases of 40% modifier solvent (MeOH or MAF) in water are compared (Figure 3A) in the flow rate range from 0.2 to 2 mL/min. The average standard deviation of HETP is 0.001 for using the mobile phase 40% MeOH and 0.0005 for using the mobile phase 40% MAF, respectively. Both the effective retention time, $t'_R = t_R - t_0$, and the bandwidth at half-height, $W_{½}$, of phenol were plotted as the function of flow rate (data not shown). Retention using the MAF mobile phase is about double that using MeOH but the peak width is slightly narrower than expected using MAF. The average asymmetry factor at 10% peak height using either 40% MeOH or MAF in water as a mobile phase, as a function of flow rate were also calculated. Peak asymmetry values using MeOH are primarily in the 1.2–1.3 range while those for MAF are consistently about 1.2. This explains the better HETP values for MAF of about a factor of 1.7.

One plausible explanation is that MAF works as silanol suppressor for the silica based C18 column. To confirm this possibility of MAF as a silanol suppressor, similar measurements and calculations for the van Deemter plot of 50 ppm phenol in water were performed using a polystyrene-divinylbenzene PRP-1 column with 60% of organic solvent (MAF or MeOH) in water as the mobile phase (Figure 3B). As expected, MeOH as a mobile phase modifier indicated a lower HETP profile as compared with MAF [12]. Since the PRP-1 column is polymeric, there is no advantage using MAF for free silanol suppression. The difference in the diffusion coefficient of the solute in the mobile phase, D_m, for MeOH and MAF is evident due to the difference in their viscosity as noted previously for EAF and MeOH (10). Since D_m is so small in a liquid and the B term in the van Deemter equation is proportional to D_m, the longitudinal diffusion term is not a major factor of the HETP plot. At higher flow rates, this difference in the diffusion coefficients will affect the resistance to the mass transfer of the solute from the bulk of the mobile phase to the surface of the stationary phase since D_m is inversely proportionally to the $C_m\mu$ term.

3.2.2 Chromatograms using MAF as a mobile phase component with UV detection—The separation of caffeine, p-nitroaniline, and phenol was first tried using 60% and 70% MAF in water as the mobile phases with the Synergi C12 column. The flow rate for the mobile phase 70% MAF in water was adjusted to 0.5 mL/min because of the high column back pressure due to the increased volume percent of MAF in water, whereas the flow rate used for 60% MAF in water as a mobile phase is 0.7 mL/min. Baseline peak resolution was evident in both chromatograms. Retention times of 8.9, 10.2, and 13.8 min were found respectively for caffeine, p-nitroaniline, and phenol using the 70% MAF mobile phase, and they increased by about a factor of 1.5–1.8 to 13.4, 18.3, and 21.1 min for the same three compounds using the 60% MAF mobile phase.

A similar separation of the same sample mixture was also carried out using the Polaris C18 column with compositions of the mobile phases of 70% (Figure 4A), 60% (Figure 4B), and 40% (Figure 4C) MAF in water with a flow rate at 1.0 mL/min. The control of peak resolution by varying the %MAF in the mobile phase is evident; baseline resolution of these three sample components in a reasonable analysis time is noted at 60% MAF. This column is more suited for the use of MAF as a modifier than the more hydrophobic Synergi MAX RP C12 column with a high surface area and stationary phase coverage causing excessive analysis times as described previously.

A similar separation of caffeine, p-nitrophenol, and phenol was done using 40% MAF-60% water on the Aqua C18 column. Using the same mobile phase composition but 40% 2M methylammonium acetate (MAA) in water and the same Aqua C18 column, there was no elution of these compounds even after 20 min. When the mobile phase was changed to 100% MeOH, large unretained peaks were quickly evident on the detector output. The solvation

abilities of the IL MAF and a methylammonium acetate buffer solution for the C18 stationary phase are quite different.

Enhanced HPLC retention of some nitroaromatics such as nitrophenols has been reported previously using an alkylammonium nitrate IL mobile phase [8]. The separation of nitrofuran drugs with similar chemical structures such as furazolidone (FZD; pKa = 5.12) and nitrofurantoin (NFT; pKa = 7.2) using 20% MAF in water as the mobile phase is shown in Figure 5A, with baseline separation at 19.6 for NFT and 21.8 min for FZD. When using 20% MeOH in water as the mobile phase with the same chromatographic conditions and sample preparation, there is no separation, with a single peak only at 9.0 min (Figure 5B). When using 10% MeOH in water as the mobile phase, the peak separation for NFT and FZD appeared very closely with no baseline separation at 28.0 min and 29.1 min, respectively (Figure 5C).

The significance of these results is that nitrofurans are known to have antimicrobial activity and their use is prohibited in feeds for food-producing animals, such as poultry and cultured fish, by the European Union (EU) and the US Food and Drug Administration (FDA) [19]. The global problem of food products contaminated by residues of the banned carcinogenic nitrofuran drugs has prompted research into how such residues accumulate in tissues from animals exposed to either a dietary or an environmental source of contamination [20].

The separations of a vitamin mixture using 5% MAF or MeOH in water as the mobile phase are compared in Figure 6. The retention times for pyridoxine, thiamine, and nicotinamide are 8.3, 9.5, and 10.3 minutes using 5% MAF in water as the mobile phase but only two components (pyridoxine and nicotinamide at 8.0 and 10.5 min) are obvious using 5% MeOH in water as the mobile phase. The thiamine containing a positively charged nitrogen on the five-membered ring strongly interacted with the silanol of the C18-based column [21] which caused poor separation due to tailing when using 5% MeOH in water as the mobile phase. The chromatogram using 5% MAF in water shows excellent peak shapes which provides additional evidence that MAF is a good silanol suppressor for silica based C18 column.

3.2.3 MAF as a mobile phase component in LC-MS—To the best of our knowledge, the use of % levels of any IL in the mobile phase has not been reported before for LC-MS. Total ion charge (TIC) as the function of the percent of organic solvent in water was first measured using liquid chromatography and ion trap mass spectrometry (LC-MS). The TIC rises modestly as the percent of MAF in water increases from 1 to 10% but then increases more rapidly at 15 and 20% (Figure 7). As expected, the TIC remains constant as the percent of MeOH increases (Figure 7). However compatibility of MAF with LC-MS is still expected up to at least 20%.

Separations of the vitamin mixture containing 100 ppm of pyridoxine, thiamine, and nicotinamide in water using 5% MAF or MeOH in water as the mobile phase were done using LC-MS with an APCI positive ion source (Figure 8). The UV chromatogram has the same separation sequence at 8.6, 9.7, and 10.6 minute for pyridoxine, thiamine, and nicotinamide, respectively, as expected. The corresponding TIC trace for pyridoxine and thiamine were shown. However, the nicotinamide molecular ion mass peak was clearly missing. One explanation for this could be the possibility of complex formation between the IL MAF and the nicotinamide ion. The compound mass chromatogram for pyridoxine showed three fragmented ion mass peaks at 134, 152, and 170, whereas for thiamine, the fragmented ion mass peaks showed up at 265, 144, and 122. The corresponding extracted ion chromatograms (EICs) showed the molecular ion mass peaks for pyridoxine at 170 and for thiamine at 265.

In Figure 9, the LC-MS with APCI separation of the same sample mixture using 5% MeOH in water as the mobile phase was poor but evidence for nictoinamide is present. As seen in both

the UV chromatogram and the TIC trace, the nicotinamide molecular ion mass peak $[M+H]^+$ = 123.0 at 10.4 minute is prominent and also shown in both the corresponding EIC trace and the compound mass chromatogram. The peaks for pyridoxine and thiamine were not separable in both TIC and the UV chromatograms. The corresponding EICs and compound mass chromatograms for the molecular ion mass peaks of pyridoxine (170.0) and thiamine (265.1) are shown at 8.1 min. and 9.9 min. in Figure 9. The mass spectra for pyridoxine and thiamine using the MAF mobile phase (Figure 8) are quite comparable to those found using MeOH (Figure 9).

A similar separation for the same vitamin mixture containing pyridoxine, thiamine, and nicotinamide in water using 5% MAF in water as the mobile phase was carried out using LC-MS with the ESI ion source positive polarity and similar chromatographic conditions (Figure 10). The UV chromatogram, TIC, EICs, and compound mass chromatograms show retention times of 29.5 min, 32.9 min, and 37.0 min for pyridoxine, thiamine, and nicotinamide, respectively. The slower flow rate was applied due to the condensation built up in the electrospray chamber when using ESI with the relatively lower drying gas temperature at 365° *C* comparing with the temperature 450°*C* used for APCI ion source. The mass spectra taken using MAF in Figure 10 are similar with respect to the major peaks in Figure 9 using MeOH. However, there is evidence of more background peaks, particularly for nicotinamide in Figure 10. The condensation built-up using ESI ion source as well as the long analysis times could be corrected using narrow bore columns and/or slower flow rates.

4. CONCLUSION

The IL methylammonium formate (MAF) has been synthesized under carefully controlled conditions and characterized with respect to viscosity and polarity. The viscosity of 9.05 cP for MAF may be the lowest reported viscosity for any IL to date. Reichardt dye and Kamlet-Taft polarity measurements show MAF is more polar than other short chain AAFs, similar to water with respect to polarizability, and quite similar to MeOH with respect to hydrogen bond acidity and basicity. Chromatographic characterization of MAF as a major mobile phase modifier showed effective suppression of peak broadening due to silanol interaction and better separation of nitrofuran drugs than MeOH. Compatibility of MAF with LC-MS is shown for both APCI and ESI modes, particularly at modest flow rates. Based on our recent spectroscopic work showing the capability of proteins when dissolved in MAF to stay in their native form [22], this LC study using MAF to separate small molecules should facilitate the application of MAF as a mobile phase modifier for the separation of proteins.

Acknowledgments

Financial support from a NIH AREA grant is very much appreciated.

References

1. Stalcup AM, Cabovska B. J Liq Chromatogr Rel Technol 2004;27:1443.
2. Chen X, Qi S. Curr Anal Chem 2006;2:411.
3. Shamsi S, Danielson ND. J Sep Sci 2007;30:1729. [PubMed: 17623452]
4. Berthod A, Ruiz-Angel MJ, Carda-Broch S. J Chromatogr A 2008;1184:6. [PubMed: 18155711]
5. He L, Zhang W, Zhao L, Liu X, Jiang SJ. J Chromatogr A 2003;1007:39. [PubMed: 12924549]
6. Berthod A, Ruiz-Angel MJ, Hugent S. Anal Chem 2005;77:4071. [PubMed: 15987112]
7. Poole CF, Kersten BR, Ho SSJ, Coddens ME, Furton KG. J Chromatogr 1986;352:407.
8. Shetty PH, Youngberg PJ, Kersten BR, Poole CF. J Chromatogr 1987;411:61.
9. Dotlich, EM. MS Thesis. Miami University; 2008.
10. Waichigo MM, Hunter BM, Riechel TL, Danielson ND. J Liq Chromatogr Relat Tech 2007;30:165.

11. Waichigo MM, Danielson ND. J Sep Sci 2006;29:599. [PubMed: 16605076]
12. Greaves TL, Weerawardena A, Fong C, Krodkiewska I, Drummond CJ. J Phys Chem B 2006;110:22479. [PubMed: 17091990]
13. Spectra Database for organic compounds SDBS wetsite http://www.aist.go.jp/RIODB/SDBS for N-methylformamide spectra.
14. Miller, JM. Chromatography: Concepts and Contrasts. Wiley; New York: 2005. p. 238
15. Reichardt C. Chem Rev 1994;94:2334.
16. Poole CF. J Chromatogr A 2004;1037:49. [PubMed: 15214660]
17. Kamlet MJ, Abboud JL, Taft RW. J Am Chem Soc 1977;99:6027.
18. Reta M, Cattana R, Silber JJ. J Sol Chem 2001;30:237.
19. Barbosa J, Moura S, Barbosa R, Ramos F, da Silverira MIN. Anal Chim Acta 2007;586:359. [PubMed: 17386735]
20. McCracken RJ, Van Rhijn JA, Kennedy DG. Food Add Contam 2006;22:567.
21. Dong MW, Lepore J, Tarumoto T. J Chromatogr 1988;442:81. [PubMed: 3417836]
22. Wei, W.; Grossman, S.; Waichigo, M.; Danielson, ND. presented at the 32nd International Symposium on High Performance Liquid Phase Separations and Related Techniques; Baltimore, MD. 10–16 May 2008; abstract P-2219-W

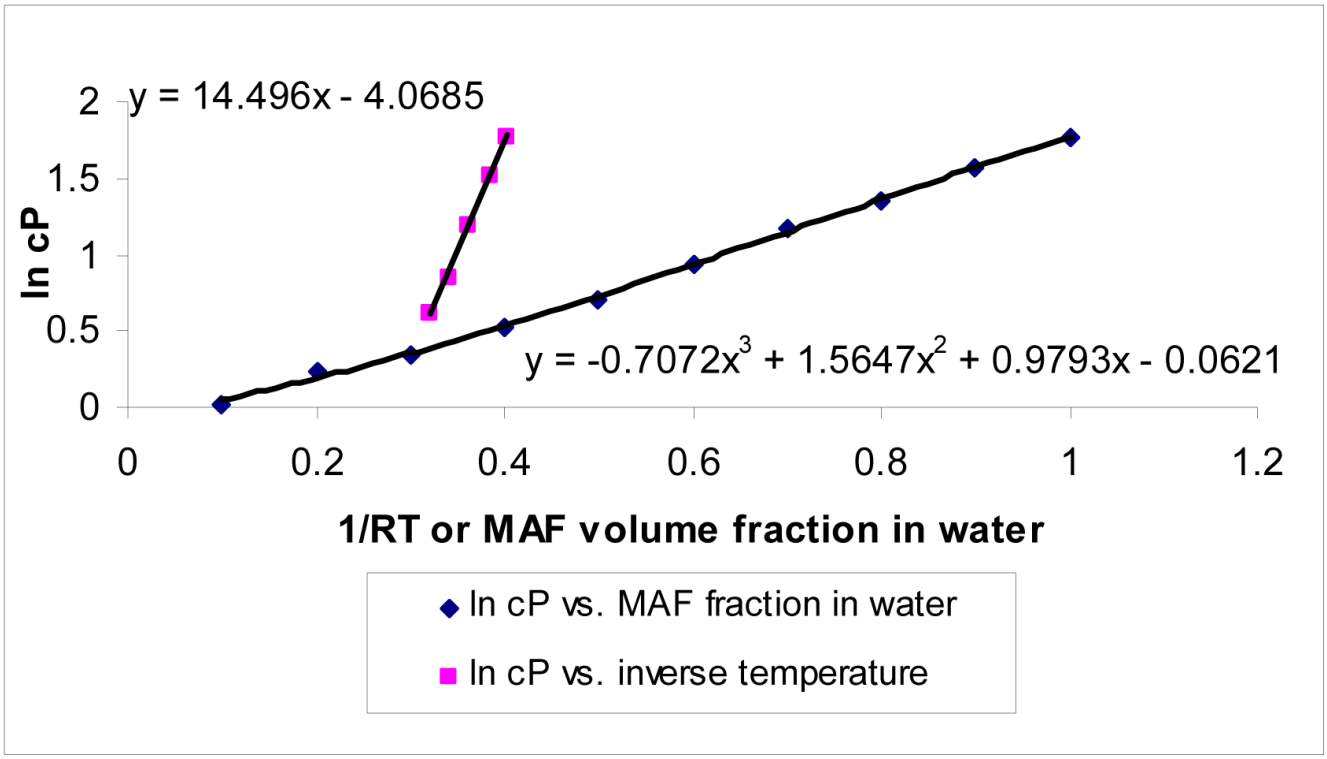


Figure 1.
Plot of ln viscosity of MAF as a function of the inverse temperature (n=3; Linear fit ■). Plot of ln viscosity as a function of volume percent of MAF in water at room temperature (n=3; polynomial fit ◆).

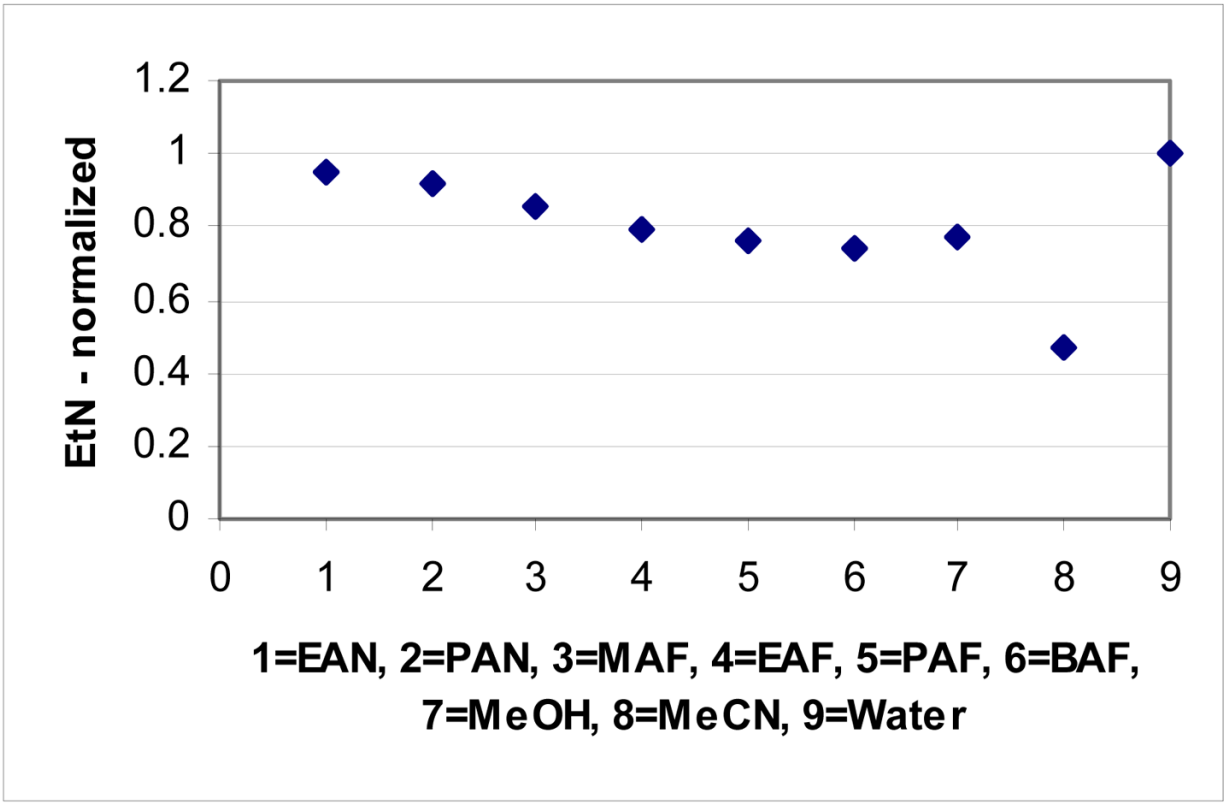


Figure 2.

The normalized polarity, E_t^N of Reichardt's dye using conventional organic solvents [MeOH, MeCN, and water (Ref. 16)] and ILs [EAN, PAN (Ref. 16)], and alkylammonium formates: [MAF(this work), EAF, PAF, BAF (Ref. 10]).

Figure 3A

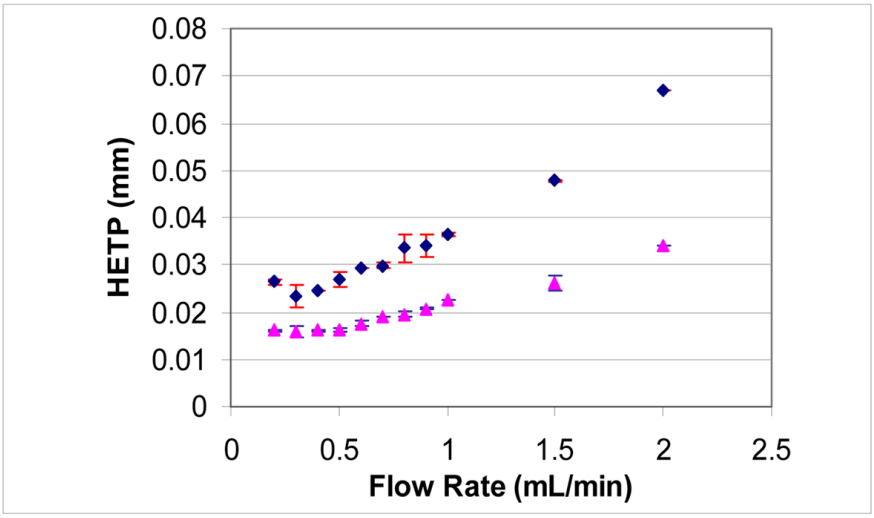


Figure 3B

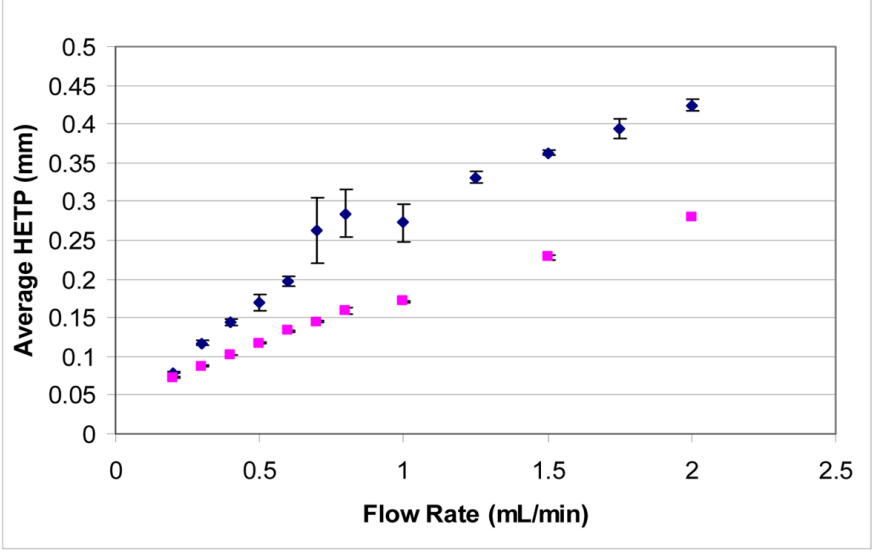


Figure 3.

(**A**) Comparison of van Deemter plots for phenol (50 ppm in water) using a mobile phase of 40% MeOH (◆) or MAF (▲) in water and the Aqua C18 column. Each data point is an average of triplicate measurements. (**B)** Van Deemter plot of phenol (50 ppm in water) using the PRP-1 column with 60% organic solvent (MAF◆ or MeOH■) in water as the mobile phase and UV detection at 270 nm. The first half of the data points (from the flow rates 0.2 up 0.8 mL/min) and the second half (from the flow rates 1.0 up 2.0 mL/min) of the data points using 60% MAF in water were measured on two separate days.

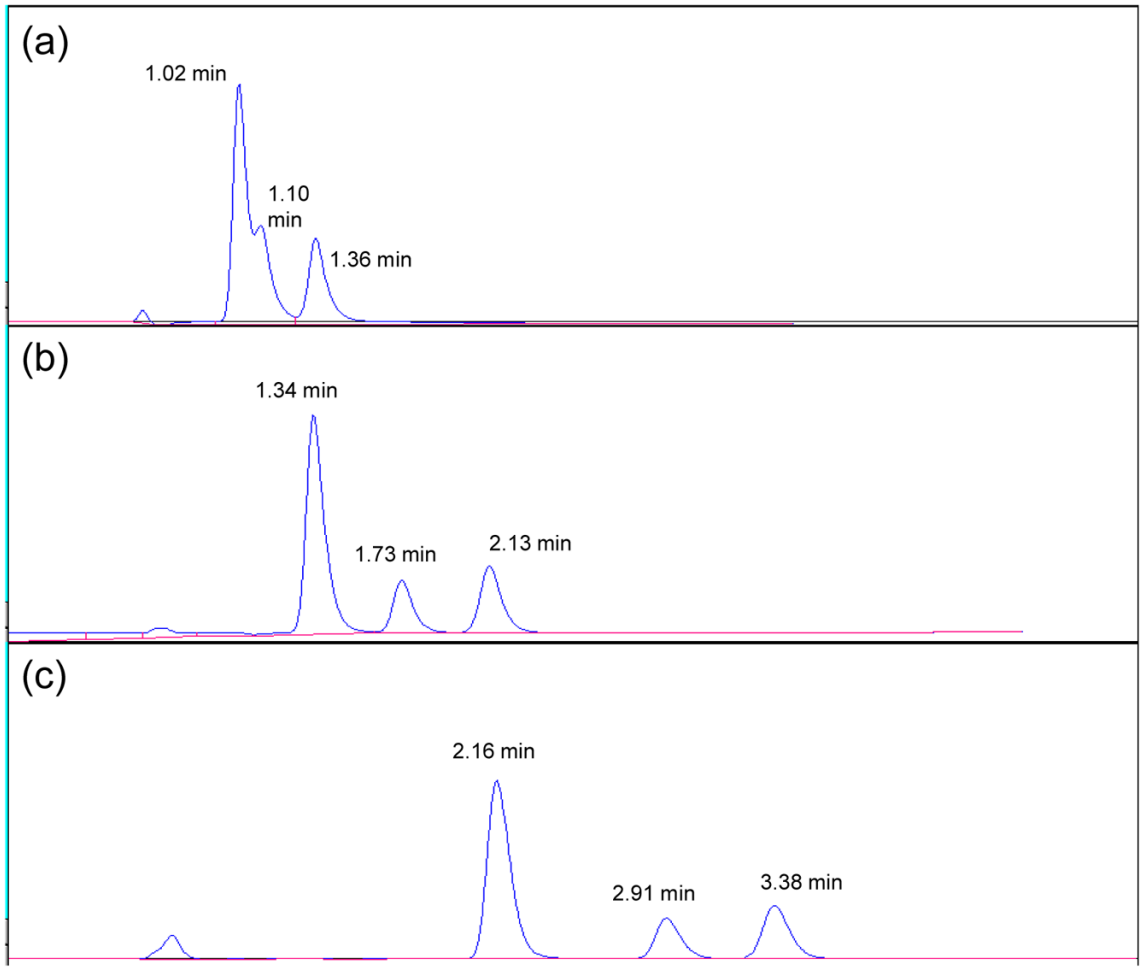


Figure 4.
Separation of the mixture containing (from left to right): 1. caffeine, 2. p-nitroaniline, 3. phenol by the Polaris C18 column with UV detection at 270 nm and the full absorbance scale at 0.08 AU, using the mobile phase of either (a) 70%, (b) 60%, or (c) 40% MAF in water at a 1.0 mL/min flow rate.

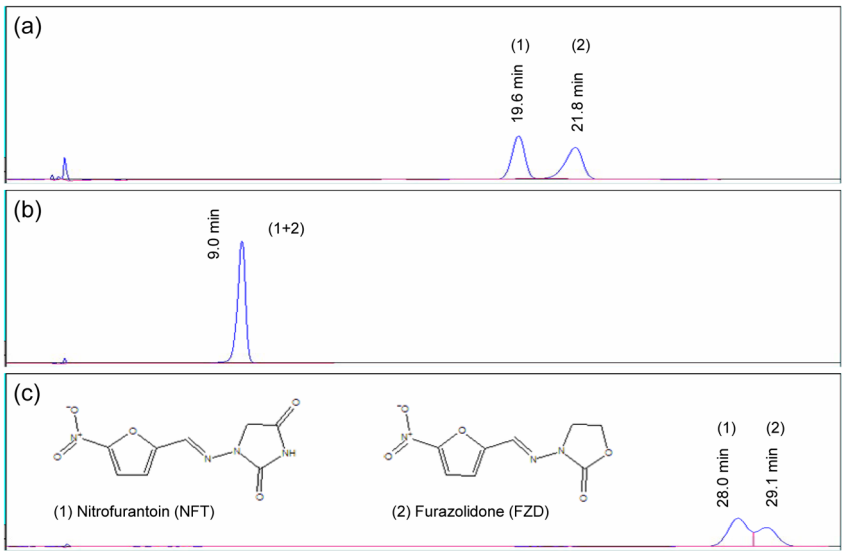


Figure 5.

Separation of the mixture containing (1) NFT and (2) FZD (30 ppm mixture prepared in 50/50 MeCN/water) using a mobile phase of either (a) 20% MAF in water, (b) 20% MeOH in water, or (c) 10% MeOH in water by the Aqua C18 column at a flow rate of 1 mL/min with UV detection at 365 nm.

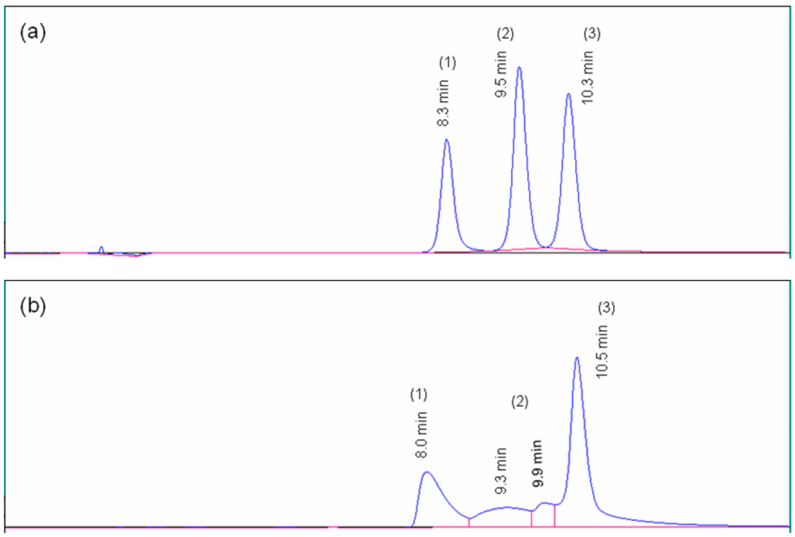


Figure 6.
Separations of the vitamin mixture (100 ppm in water) containing (1) pyridoxine, (2) thiamine, and (3) nicotinamide using (a) 5% MAF in water and (b) 5% MeOH in water. Chromatographic conditions: Aqua C18 5μ column, flow rate 0.7 mL/min, UV detection 254 nm at the full absorbance scale at 0.8 AU.

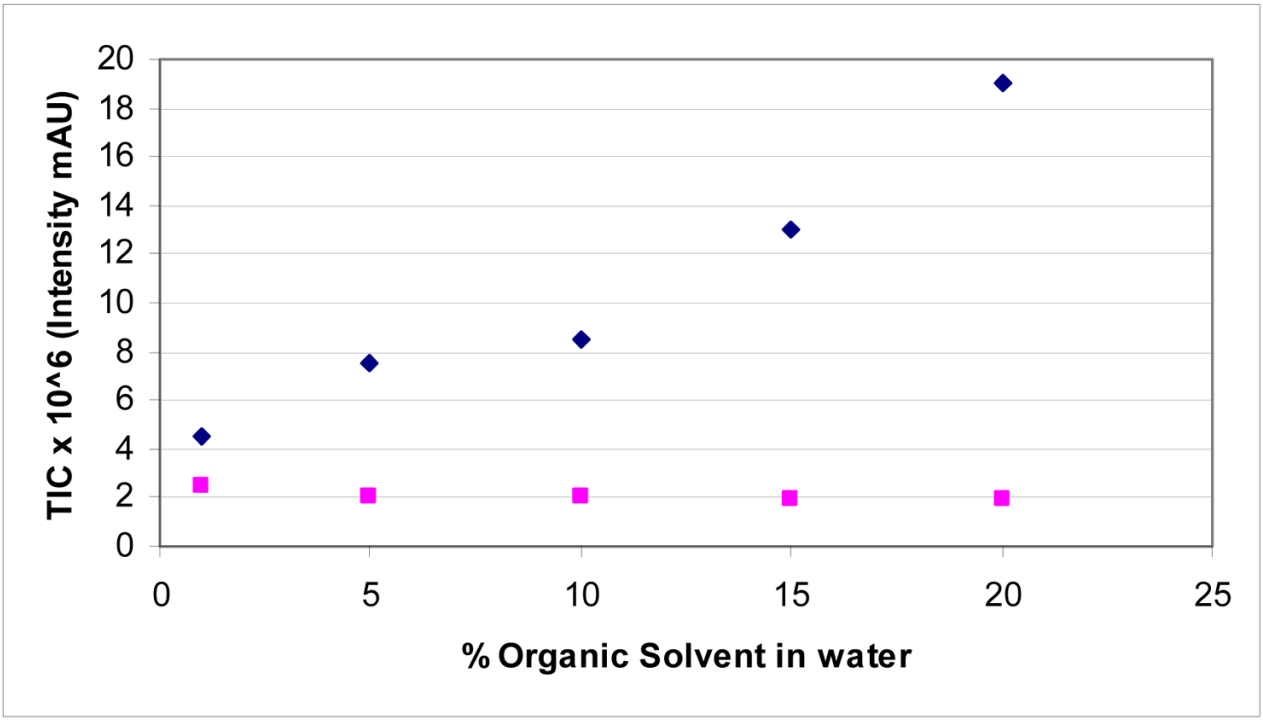


Figure 7.
Total ion charge (TIC) as a function of % organic solvent (MAF◆ or MeOH■) in water. Positive polarity was used with APCI as the ion source. The LC flow rate was 0.7 mL/min.

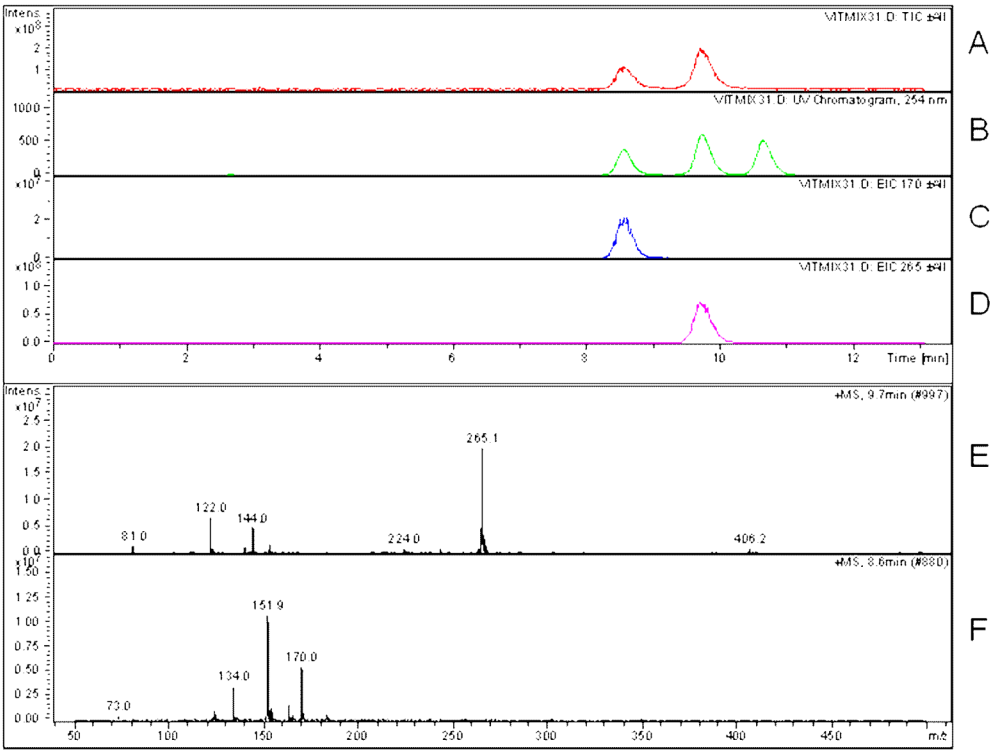


Figure 8.

LC-MS separation by APCI of pyridoxine (8.6 min.), thiamine (9.7 min.), and nicotinamide (10.6 min.), 100 ppm mixture prepared in water, using 5% MAF in water as mobile phase. Chromatographic conditions: Aqua C18 column; flow rate 0.7 mL/min, UV detection 254 nm; column back pressure 66 bar; and 50 μL injection sample size. APCI positive polarity was applied. (**A**) Total ion chromatogram (TIC); (**B**) UV chromatogram; (**C, D**) extended ion chromatograms (EIC) for pyridoxine and thiamine, respectively; (**E, F**) mass chromatograms for thiamine (265, 144, and 122 at 9.7 min) and pyridoxine (134, 152, and 170 at 8.6 min), respectively.

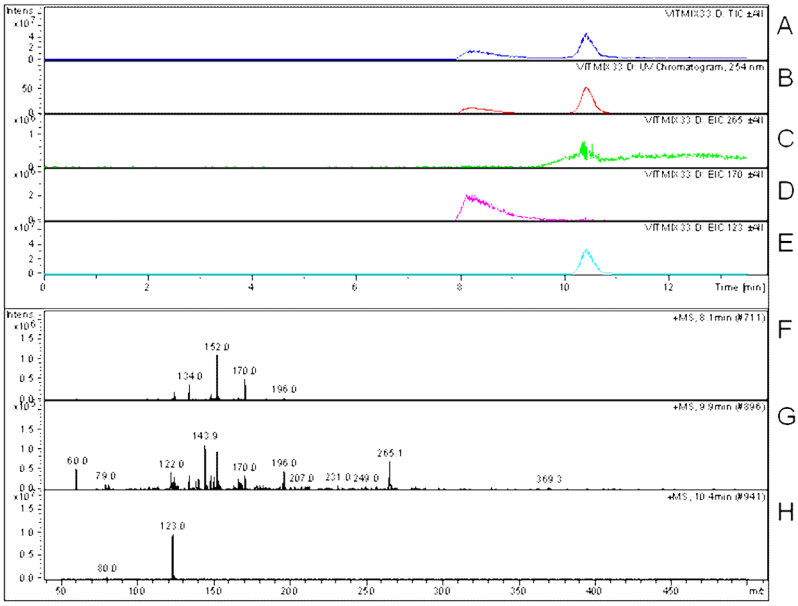


Figure 9.

LC-MS separation by APCI of pyridoxine, thiamine, and nicotinamide (100 ppm mixture in water) using 5% MeOH in water as the mobile phase. LC and MS conditions: same as in Figure 8. (**A**) Total ion chromatogram (TIC); (**B**) UV chromatogram; extended ion chromatogram (EIC) for (**C**) pyridoxine, (**D**) thiamine, and (**E**) nicotinamide, respectively; mass chromatogram for (**F**) pyridoxine (134, 152, and 170 at 8.1 min), (**G**) thiamine (265, 144, and 122 at 9.9 min), and (**H**) nicotinamide (123 at 10.4 min), respectively.

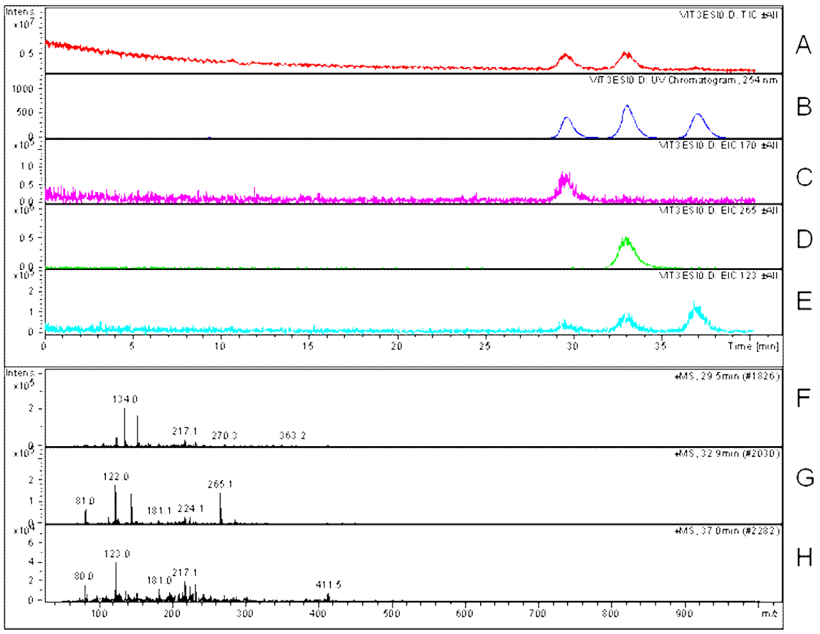


Figure 10.

LC-MS separation by ESI of pyridoxine (29.5 min.), thiamine (32.9 min.), and nicotinamide (37.0 min.) (100 ppm mixture in water) using 5% MAF in water as the mobile phase. LC conditions same as in FIgure 8. (**A**) Total ion chromatogram (TIC); (**B**) UV chromatogram; (**C, D, E**) extended ion chromatograms (EIC) for pyridoxine, thiamine, and nicotinamide, respectively; (**F, G, H**) mass chromatograms for pyridoxine (134, 152, and 170 at 29.5 min), thiamine (265, 144, and 122 at 32.9 min), and nicotinamide (123 at 37.0 min), respectively.