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Article

Increasing the Sensitivity of an LC–MS Method for Screening Material Extracts for Organic Extractables via Mobile Phase Optimization

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Organic extractables (substances extracted from materials used in pharmaceutical packaging) are discovered, identified, and quantified via screening of extracts with analytical methods including liquid chromatography with mass spectrometric detection (LC–MS). Because extractables include a large number of diverse compounds that are typically present in plastic extracts at low levels, the LC–MS methods must be broad scope and sensitive. To accomplish these objectives, screening studies typically couple gradient reversed-phase separations with electrospray MS detection (both positive and negative ion modes). While such methods are generally applicable for a number of extractables, they are not optimal for some commonly encountered extractables due to either poor chromatographic performance (e.g., peak tailing) or poor MS response. Modifications to mobile phase composition (e.g., pH adjustment) were examined to improve the performance of an LC–MS screening method. The use of 0.1% acetic acid with 1 mM ammonium acetate (pH 3.6) as the aqueous portion of the mobile phase provided favorable sensitivities for a number of extractables both in positive and negative ion modes. In positive ion mode, the acidic mobile phase improved responses for moderately weak basic compounds by increasing their degree of protonation. For very weak basic compounds such as amides, ammonium ions in the mobile phase promoted proton adduct responses. In negative ion mode, an acidic mobile phase containing acetate anion improved ESI responses for acidic compounds, primarily due to gas phase effects.

Introduction

Plastic materials are widely used in systems for packaging and delivering medical products, such as solution containers, transfusion sets, transfer tubing, and devices. Extractables are compounds, present in these plastic materials, which could leach into the medical products under normal conditions of use. Both the identities of the extractables and their accumulation levels may affect the plastic material's ultimate compatibility with the medical product (1). Given the large number of compositionally diverse plastic materials used in pharmaceutical packaging and devices, extractables include a wide range of compounds with many different structures such as antioxidants and their degradants, molding agents, monomers and oligomers, plasticizers, curing agent for elastomers, residual polymerization initiators and catalysts, and reaction products typically formed during the plastic's harsh processing conditions (2, 3).

Screening is the term applied to the process of chemically characterizing extracts of plastic materials for extracted substances. The screening process includes the steps of discovery (e.g., producing a response for an individual extractable), identification and quantitation. Typical approaches used in screening for organic extractables couple a chromatographic separation [e.g., gas chromatography (GC) or liquid chromatography (LC)] with sensitive, broad-scope and information-rich detection methods such as mass spectrometry (MS). (4) LC–MS methodologies are mainly used for semi-volatile and non-volatile compounds and generally couple a gradient reversed-phase separation with MS detection using electrospray ionization (ESI) in both positive and negative ion modes. Typical LC–MS methods for extractables screening utilize varied aqueous mobile phases such as ammonium acetate buffer (5), ammonium formate buffer (6), and acetic acid and formic acid (7). While mobile phase optimization for various reversed-phase LC–MS applications has been studied extensively (for example, 8-11), such studies are not specifically applicable to extractables screening. However, such optimization is especially important for extractables screening as the potential number of extractables is large and the extractables are generally present in the extracts at low levels.

In an effort to optimize mobile phase composition for extractable screening studies, basic, neutral, and acidic mobile phases were evaluated with respect to sensitivity for different commonly encountered and chemically diverse extractables. Based on this evaluation, a mobile phase system was developed which facilitates screening using both positive and negative ion detection modes. The effects of mobile phase additives and solution pH on sensitivity were studied and possible ESI mechanisms for the observed behaviors are proposed and discussed.

Experimental

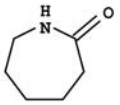
Chemicals and reagents

Twelve extractables representing three types of compounds were examined (Figure 1). Basic compounds included caprolactam (CAP), dibenzylamine (DBA), hexadecanamide (HAM), and octadecanamide (OAM). Neutral compounds included 25-crown-5 (25C5) and 30-crown-6 (30C6), and acidic compounds included benzoic acid (BA), 2-ethylhexanoic acid (EHA), Irganox degradant #2 (Irg2), 2,4-di-t-butylphenol (DBP), myristic acid (MyA), and palmitic acid (PmA).

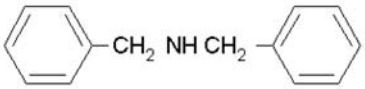
Solvents and chemicals were obtained commercially in the highest appropriate purity. Methanol (MeOH, HPLC grade) was obtained from Honeywell Burdick and Jackson (Morristown, NJ). Ammonium acetate (NH4Ac, HPLC grade), acetic acid (HAc), formic acid (HFo), ammonium hydroxide, and CAP, DBP,

A

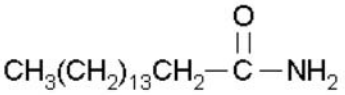
Basic Compounds.



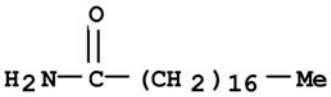
Caprolactam; CAP, CAS RN 105-60-2, $C_6H_{11}NO$, formula weight = 113.16



Dibenzylamine; DBA, CAS RN 103-49-1, $C_{14}H_{15}N$, formula weight = 197.28



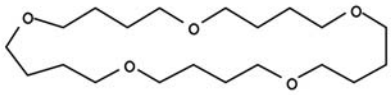
Hexadecanamide; HAM, CAS RN 629-54-9, $C_{16}H_{33}NO$, formula weight = 255.44



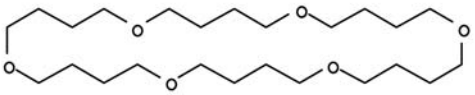
Octadecanamide; OAM, CAS RN 124-26-5, $C_{18}H_{37}NO$, formula weight = 283.49

B

Neutral Compounds.



25-crown-5 (1,6,11,16,21-Pentaoxacyclopentacosane); 25C5, CAS RN 56890-57-4, $C_{20}H_{40}O_5$, formula weight = 360.53



30-crown-6 (1,6,11,16,21,26-Hexaoxacyclotriacontane); 30C6, CAS RN 64001-05-4, $C_{24}H_{48}O_6$, formula weight = 432.63

Figure 1. Chemical Information for the Target Extractables.

Ba, EHA, MyA, PmA, and DBA were obtained from Aldrich (St. Louis, MO). HAM and OAM were from TCI (Portland, OR). Irg2, 25C5, and 30C6 were synthesized and qualified by Baxter internally. Distilled/deionized water was used throughout this study.

Solution Preparation

Individual extractables stock solutions were prepared by dissolving ~ 25 mg standard compound in a 25.0-mL volumetric flask with methanol. A single composite test mixture containing 1 ppm of each extractable in 10% methanol was prepared by serial dilution of the individual of stock solutions. Additionally, single analyte solutions were prepared by dilution of the individual stock solutions in appropriate buffer solutions. For example, 500 ppb CAP and 500 ppb HAM solutions in 1:1 MeOH/10 mM NH4Ac were prepared for infusion experiments by dilution of the corresponding stocks with the appropriate amount of methanol and 10 mM NH4Ac. 500 ppb DBA or 1 ppm BA were prepared by diluting the individual stocks into 1/1 mxtures of MeOH and aqueous buffers including water, pH 8.3, pH 6.1, pH 5.0, pH 4.0, pH 3.3 (0.1% HAc), and pH 2.7 (0.1% formic acid) solutions.

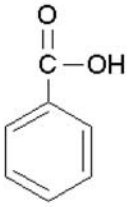
Various mobile phases were prepared by dissolution of the appropriate salts in either water or methanol followed by pH adjustment as appropriate. These mobile phases included: 1, pH 7.0 10 mM NH4Ac in water; 2, pH 8.1 (0.01% NH3 in 10 mM NH4Ac); 3, pH 5.1(0.025% HAc in 10mM NH4Ac); 4, pH 4.5 (0.1%HAc in 10 mM NH4Ac); 5, pH 3.3 (0.1% HAc); 6, pH 2.7 (0.1% HFo); 7, pH 3.6 (0.1% HAc in 1mM NH4Ac); 8, pH 3.1(0.5% HAc in 1mM NH4Ac); 9, pH 3.3 (0.01% HFo).

LC–MS Systems

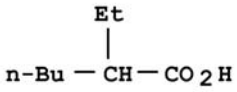
Two LC–MS systems were used. An Agilent (Santa Clara, CA) 1100LC/MSD system consisting of a binary pump (G1312A),

C

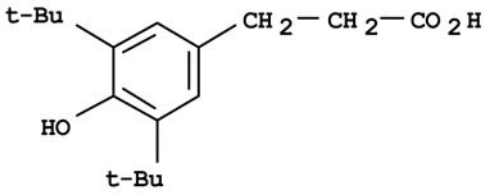
Acidic Compounds



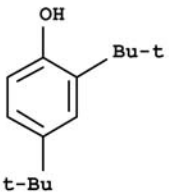
Benzoic acid; BA, CAS RN 65-85-0, $C_7H_6O_6$, formula weight = 122.12



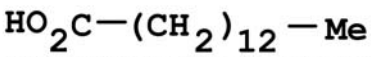
2-Ethyl-hexanoic acid; EHA, CAS RN 149-57-5, $C_8H_{16}O_2$, formula weight = 144.21



Irganox Degradate #2 (3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoic acid); Irg2,CAS RN 20170-32-5, $C_{17}H_{26}O_3$, formula weight = 278.39.



2,4-di-tert-butylphenol; DBP, CAS RN 96-76-4, $C_{14}H_{22}O$, formula weight = 206.32



Tetradecanoic (Myristic) acid; MyA, CAS RN 544-63-8, $C_{14}H_{28}O_2$, formula weight = 228.37

Hexadecanoic (Palmitic) acid; PmA, CAS RN 57-10-3, $C_{16}H_{32}O_2$, formula weight = 256.42

Figure 1. Continued.

refrigerated autosampler (G1329A. G1330B), thermostatted column compartment (G1316A), degasser (G1379B), diode array detector (G1315B), and 1100 mass detector was used for the LC–MS and flow injection experiments.

The LC–MS conditions used to study the effect of mobile phase pH are summarized in Table I. The LC–MS system was equilibrated between the various mobile phase changes. The flow injection analysis (FIA) experiments were conducted using 1:1 mixtures of MeOH and various aqueous solutions (0.8 mL/min flow rate and 100 μL injection) and the same mass spectrometer parameters as in Table I.

An Agilent ESI tuning mix solution was used to check mass spectrometer mass accuracy on a daily basis. LC–MS system stability was checked daily at the beginning and/or at the end of runs via multiple injections of appropriate standard solutions. As relatively stable peak intensities were obtained for all LC–MS experiments, it was concluded that the LC–MS instrument was operated under stable conditions.

The system used in the infusion experiments consisted of an Applied Biosystems (Foster City, CA) API4000 mass spectrometer coupled to a Hamilton infusion pump. The data was acquired and analyzed via a Dell (Round Rock, TX) Precision 390 Workstation using Applied Biosystems Analyst 1.4.2 software. Samples (500 ppb CAP and 500 ppb HAM solutions) were infused into the mass spectrometer at 10 μL/min, with ESI voltage of 5000 V, ambient source temperature, GS1 at 17, GS2 at 0, curtain gas at 10, detector CEM at 2200 v. Decluster

Table I
LC–MS Operating Conditions for Experiments Performed using Agilent LC–MSD

Operating Parameter	Operating Value	
Column	Agilent Zorbax Eclipse Plus C18, 100 x 3.0 mm, 3.5 μm particles	
Column Temperature	40°C	
Mobile Phase Components	A = various buffers in water, B = methanol	
Mobile Phase Gradient	Time	% B
	0.0	2
	8.0	95
	11.0	95
	12.0	2
Mobile Phase Flow Rate	0.8 mL/min	
Sample Size	60 μL	
Detection, MS	API-ES, positive ion and negative ion (mass range 80 – 1200)	
MS Gas Temperature	350°C	
MS Drying Gas	12 L/min	
Nebulizing Pressure	35 psig	
Capillary Voltage	Positive, 4000V; Negative, 4500V	

potentials were varied during the experiments to check the in-source fragmentation process.

Results and Discussion

General

The test mixture containing the 12 target extractables at a concentration of 1ppm each was analyzed using five different binary mobile phase systems whose aqueous component had a pH ranging from 2.7 to 8.1. Positive and negative ion scans were obtained simultaneously in a single day under stable instrument conditions. The positive and negative total ion chromatograms (TIC) of the mixture are shown in Figures 2 and 3; typical positive ion mode mass spectra for selected target extractables are shown in Figure 4. In general, all the targets produced recognizable TIC signals in the appropriate ionization mode, although it is clear that the responses (ion intensities) changed as a function of mobile phase composition (see Tables II and III). Some portion of the variation in the magnitude of the TIC responses is linked to differing distributions of analyte adducts as a function of mobile phase composition.

In addition to the change of response as a function of mobile phase pH, the retention times of the ionic targets (basic and acidic extractables listed in Figure 1) changed as well.

MS signal intensities may depend on multiple factors such as mobile phase pH and organic percentage, LC separation efficiency, ESI source parameters, and type and concentration of mobile phase electrolytes. In order to more clearly understand the effect of pH on the magnitude of the response for the varied ions, flow injection analysis (FIA) was conducted with aqueous solutions whose pH ranged from 2.7 to 8.3. In addition, a 1:1 methanol/water solution was examined. The FIA extracted ion response profiles for protonated DBA and deprotonated BA are shown in Figure 5 and 6. As the positive ions and negative ions responded differently to mobile phase composition, each is discussed in greater details as follows.

Positive ions

The positive ion spectra generally included three different adducts, $[M\text{-}H]^+$, $[M\text{-}NH4]^+$ and $[M\text{-}Na]^+$ depending on the individual analyte (for example, Figure 4). While DBA and CAP predominantly formed their protonated adducts, Irg2, 25C5, and 30C6 preferentially formed ammoniated adducts while the HAM and OAM spectra included both protonated and sodiated adducts.

In considering the effect of mobile phase composition on the responses of the positive ion adducts, we will focus on protonated and ammoniated adducts, as ammoniated adducts play an important role for mobile phases containing ammonium ion additives.

Mobile phase pH effect on protonated adducts

The formation of protonated adducts is closely linked to mobile phase pH as lower pH provides more available hydronium ion in solution. It has been generally reported that the lower the pH of the mobile phase, the larger the response of the protonated adduct is for weak bases as strong acidity could enhance the solution's proton abundance (13–14). However, it is not easy to quantitatively link a compound's basicity with the response of its protonated adduct and wrong-way-around phenomena complicate this issue (11). In this study we considered bases of varying strength (relatively strong base DBA, weak base CAP, and very weak base HAM) to investigate the relationship between solution pH and protonation intensity.

The relatively strong base DBA has a pK_b of 5.24 in water (15) and exhibited varied LC–MS retention times as a function of mobile phase pH, as seen in Figure 2. Under FIA conditions, DBA predominantly formed the protonated adduct; a decrease in solution pH resulted in an increase in the response of the $[M\text{-}H]^+$ adduct (Figure 5). It is noted that in pure methanol/water DBA exhibited a high proportion of protonated adducts, similar to the behavior shown in the pH 2.7 (0.1% formic acid) solution. This behavior could be due to the increase of droplet acidity resulting from ESI tip electrochemical reactions and fewer electrolytes competing with the target analytes in the droplet for gas phase ionization. For example, the following electrochemical reaction

$$4OH^-(aq) \rightarrow O_2(g) + 2H_2O + 4e^-(\text{on metal surface})$$

was ascribed to the droplet acidity increase in positive ESI mode under high ESI tip voltage, as the hydroxyl ions are removed from the solution (12). The extra-positive charges mostly reside on the droplet surface because of electrostatic forces, making surface acidity higher than bulk solution of the droplets. When no buffer is present in the droplets, droplet pH will be more susceptible to change.

Alternatively, DBA exhibited a slightly decreased response when mobile phase pH dropped from 8.1 to 5.1, as seen in Figure 2 and Table II. This behavior can be explained by changes in DBA's retention time as a function of mobile phase pH. At low pH, DBA, in its ionic form, eluted early in the mobile phase gradient, where the mobile phase is primarily aqueous. In such an aqueous environment, DBA's response factor has been reported to decrease. (16) Thus the competing factors of mobile phase acidity and reduced retention times dictate the behavior of relatively strong bases such as DBA.

Caprolactam (CAP) is a weak base with pK_b about 15.5 in water (15). The flow injection analysis of CAP in varied pH

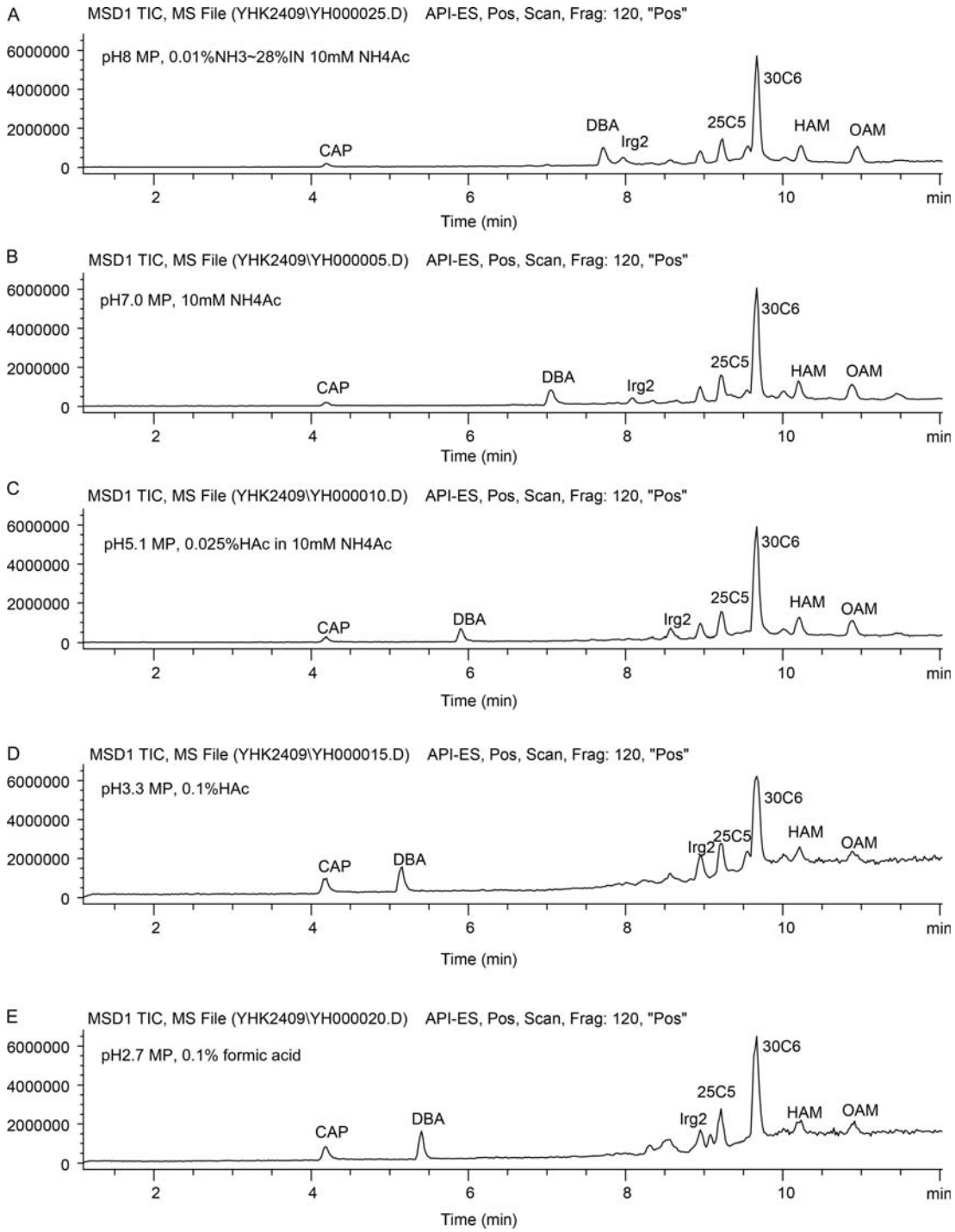


Figure 2. Positive Ion ESI LC–MS analysis of the standard mixture using conditions in Table I with varied aqueous mobile phases: (A) pH8.1 0.01% NH3 in 10 mM NH4Ac; (B) pH7.0 10 mM NH4Ac; (C) pH5.1 0.025% HAc in 10mM NH4Ac; (D) pH3.3 0.1% HAc; (E) 0.1% formic acid. Mobile phase pH affected the adduct formation and distribution of several analytes, producing a corresponding difference in peak area response (see Table II). Additionally, the retention times of the ionic analytes (e.g., DBA and Irg2) varied as a function of mobile phase pH.

solutions did not produce only protonated adducts; rather, multiple peaks including the sodiated monomer and dimer were observed at high intensities. Nevertheless, one expected that the response of the $[M\text{-}H]^+$ adduct would increase with decreasing mobile phase pH. Unlike DBA, whose retention was pH dependent, CAP retention is largely pH-independent and thus the pH effect on CAP's MS response can be observed without complications due to shifting retention times. The expectation was realized as the $[M\text{-}H]^+$ ion intensity increased with decreasing mobile phase pH (Table II).

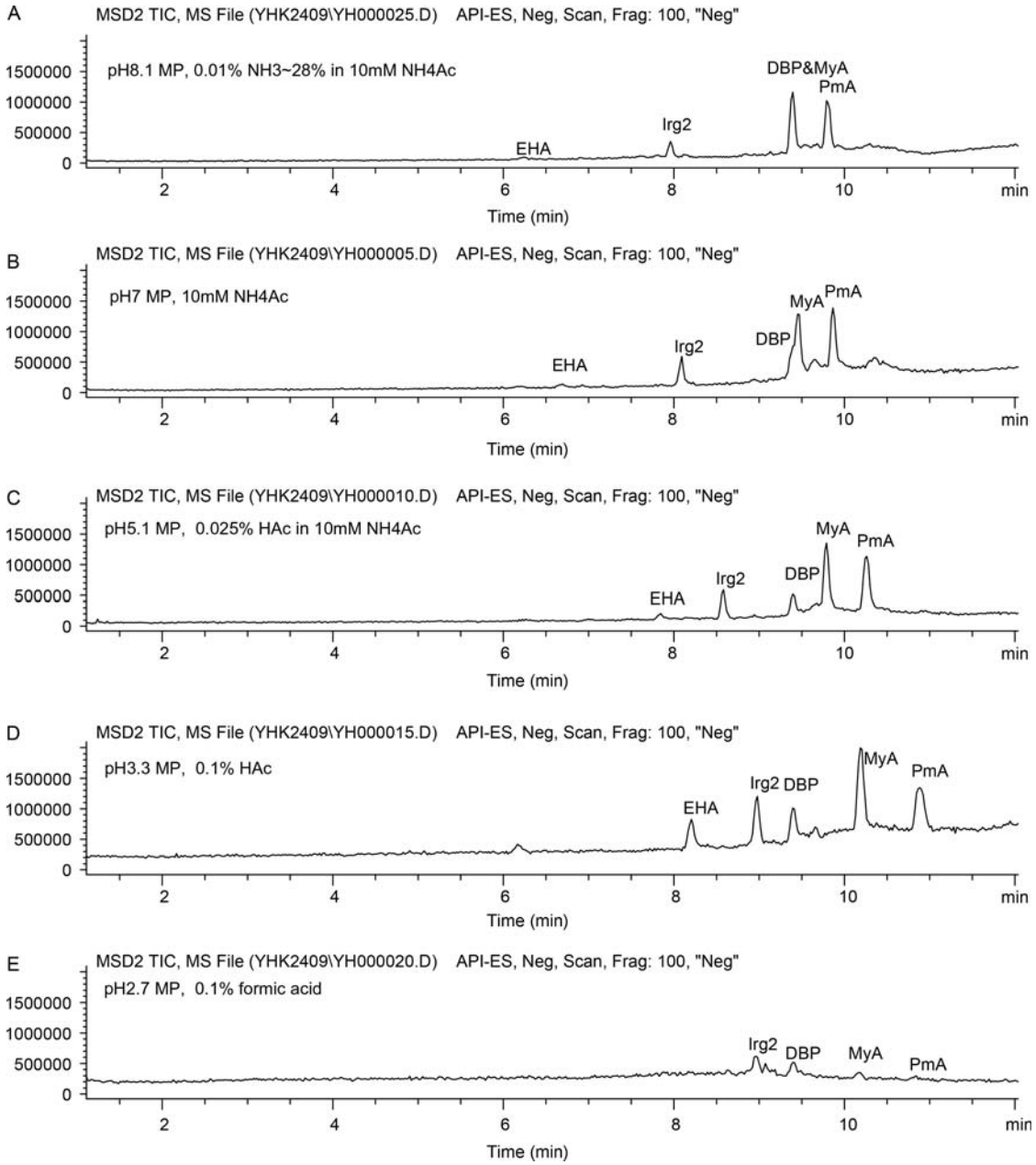


Figure 3. Negative Ion ESI LC–MS analysis of the standard mixture using conditions in Table I with varied aqueous mobile phases: (A) pH8.1 0.01% NH3 in 10 mM NH4Ac; (B) pH7.0 10 mM NH4Ac; (C) pH5.1 0.025% HAc in 10mM NH4Ac; (D) pH3.3 0.1% HAc; (E) 0.1% formic acid. Mobile phase pH affected the adduct formation and distribution of several analytes, producing a corresponding difference in peak area response. Additionally, the retention times of the ionic analytes (e.g., BA, Irg2, MyA, PmA) varied as a function of mobile phase pH.

Hexanedecanoamide (HAM) is estimated to be a very weak base (pK_b >15.5) and its primary amide structure should make it a more weak base than CAP, which is a cyclic secondary amide. The additional alkyl group in CAP make protons more attractive to nitrogen site. Similar to CAP, HAM mainly formed protonated and sodiated adducts in FIA, as seen in Figure 4.

Although one would expect HAM (and OAM) to behave similarly to CAP in LC–MS, the intensity of the protonated adducts of HAM and OAM were lower in low pH buffers (pH 3.3 and pH 2.7) than in higher pH buffers (Figure 2 and Table II), despite the fact that their retention times were constant under varied mobile phase conditions. Further analysis indicated that ammonium ions in higher pH buffers might play an important role in the formation of the protonated adducts. The increased intensity of the protonated adduct in the mobile phases that contained ammonium ions may indicate a specific pathway for protonated adduct formation for the amide type of compounds. It is proposed that amides may first attach to ammonium ions and then lose ammonium to form protonated adducts in the ESI gas phase, a process that was reported by Draper et al. (17) at low collision energy for microcystin toxins.

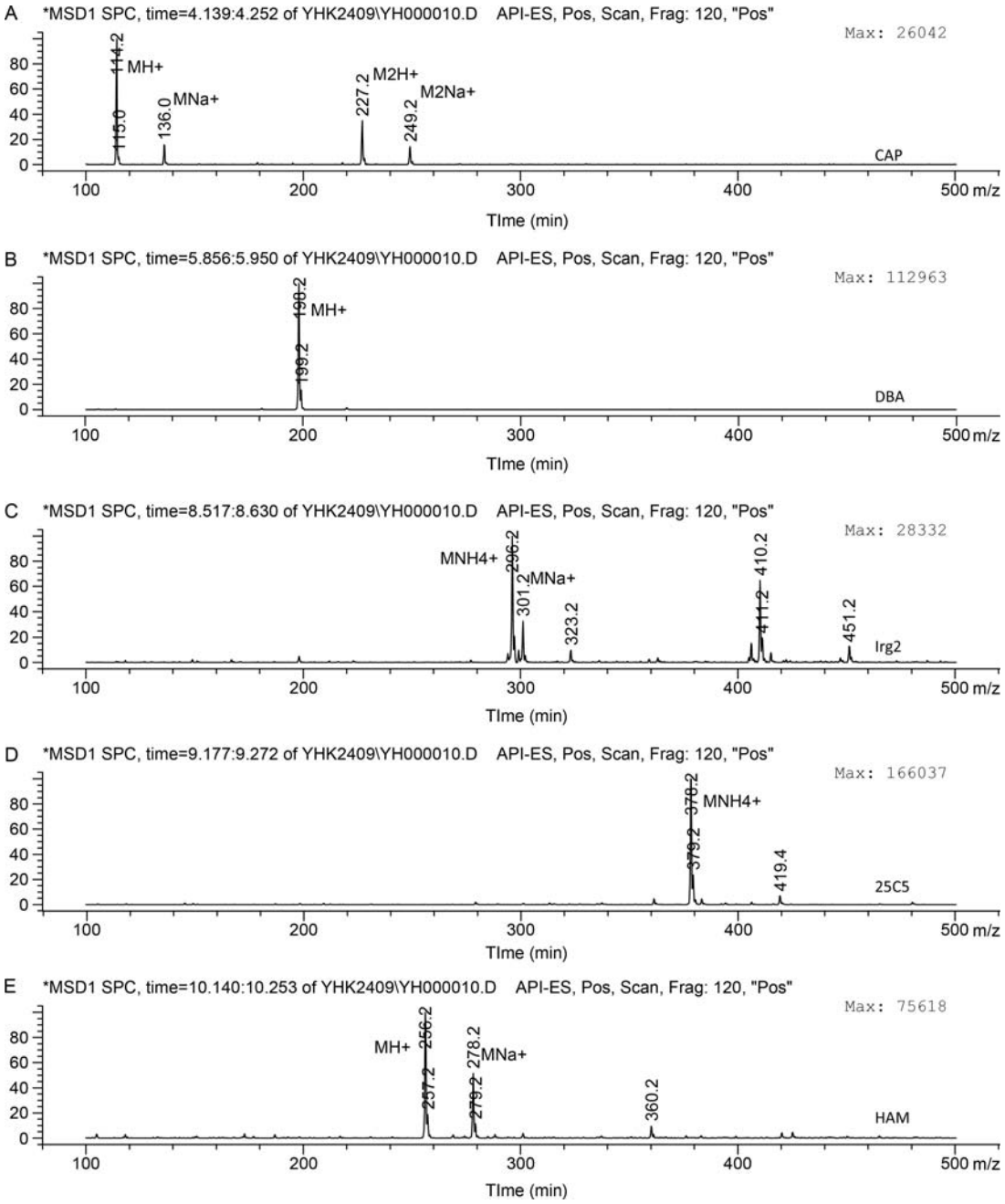


Figure 4. Positive ion mass spectra obtained for several target extractables using mobile phase C from Figure 2. The illustrated spectra represent the average of at least three spectra obtained at various points on the analyte peaks. Extractables shown include (A) CAP; (B) DBA; (C) Irg2; (D) 25C5; (E) HAM. The spectra illustrate the distribution of adducts for each of the extractables. Relatively strong base analytes, such as DBA, form predominately protonated adducts while weak or non-base analytes such as 25C5 form predominately ammonium adducts.

To investiagte the ammonium adduct intermediate mechanism, 500 ppb HAM was infused in 1:1 MeOH/10mM NH4AC solution and responses were measured at varied decluster potentials using the API 4000 mass spectrometer. The results of this experiment are shown in Figure 7. At a low DP of 20 V, the ammonium adduct of m/z 273 was present as the base ion, accompanied by protonated and sodiated adducts. When DP increased, the response for the ammonium adduct decreased and eventually disappeared at DP 120 V, while the response for the protonated adduct increased accordingly. This behavior demonstrates that a majority of the protonated adduct was converted from ammonium adduct at DPs between 80 to 120 V. When a similar set of experiments was conducted with CAP, the intermediate ammonium adduct was also observed, as seen in Figure 8. It is noticed that the response for the ammonium adduct for CAP is much weaker than the response for HAM while the response for the protonated adduct was stronger than that for HAM. The reason for this trend could be the

Table II
Peak Areas, Total Ion Current (TIC) and Extracted Ion Current (EIC) Responses, for Selected Target Compounds in Various Mobile Phases (Positive Ion Mode). In the case of the EIC responses, the m/z ratio of the adduct used is indicated

MP pH	Response in counts x 10^6											
	Cap		DBA		25C5	30C6	HAM			OAM		
	TIC	$[M-H]^+$, m/z114	TIC	$[M-H]^+$, m/z198	TIC[1]	TIC[1]	TIC	$[M-H]^+$, m/z256	$[M-Na]^+$, m/z278	TIC	$[M-H]^+$, m/z284	$[M-Na]^+$, m/z306
8.1	0.915	0.542	5.20	4.96	5.22	25.8	4.44	2.16	0.899	5.38	2.75	0.115
7.0	0.934	0.687	4.92	4.35	6.01	26.9	4.31	2.16	0.730	4.90	2.49	0.947
5.1	1.23	0.743	3.30	2.90	0.607	27.1	4.84	2.02	1.04	5.04	2.11	1.33
3.3	4.25	2.36	6.67	6.21	7.24	27.1	3.63	0.342	1.62	3.59	0.294	1.67
2.7	4.05	2.86	6.59	5.47	8.54	27.1	3.85	1.17	1.40	3.90	0.960	1.37

Note: [1]The responses for the $[M-NH_4]^+$ adducts for these compounds showed the same trend as the TIC.

Table III
Peak Areas, Extracted Ion Current (EIC) Responses, for Selected Target Compounds in Various Mobile Phases (Negative Ion Mode, $[M-H]^-$ ions)

Mobile	Response in counts × 10^6					
Phase pH	BA m/z 121	EHA m/z/143	Irg 2 m/z 277	DBP m/z 205	MyA m/z 227	PmA m/z 255
8.1	0.000	0.159	0.723	1.02	1.97	2.33
7.0	0.000	0.312	1.23	1.71	2.34	3.06
5.1	0.028	0.399	1.19	1.25	2.64	2.75
3.3	0.243	1.00	2.08	1.93	2.97	2.40
2.7	0.011	0.012	0.676	0.264	0.189	0.175

basicity difference between HAM and CAP that was mentioned previously. The higher basicity of CAP increases the ability to form protonated adducts while decreasing the ability to form ammonium adducts. For the meta-stable HAM ammonium adduct, low amide basicity coupled with the presence of a carbonyl function group were proposed to facilitate adduct formation and transform from $NH4^+$ to hydronium ion.

OAM has similar structure as HAM and exhibited similar LC–MS adducts behavior as seen in Figure 2 and Table II.

The alternative protonation pathway for these very weak bases could be utilized to interpret literature data provided by Ikonomou etal and Ehrmann et al. (13, 14). While their data for very weak bases did not fit for the trend of decreasing response for the protonated adducts with increased the pK_b values, such a trend could be readily explained by our proposed ammonium facilitated protonation pathway, as ammonium ions are always present in reagent grade solvents (18) at low levels and could help protonation of very weak bases in the acidic solutions. This mechanism could also help to interprete some wrong-way-around phenomena observed by different researcher groups (11, 19–21). For example, it was reported that some weak bases (atrazine pKa 1.7, irgarol pKa 4.9) do not exhibit significantly different ESI protonation responses over a pH ranging from 9.0 to 2.5 (11). This trend could be linked to the ammonium facilitated protonation pathway, so that the proton adduct responses could be less dependent on the solution pH.

Formation of ammoniated adducts

Ammoniated adducts are commonly formed by many compounds that lack strong basic functional groups (9). In our test mixture, Irg2, 25C5, and 30C6 all produced strong ammonium adducts under the studied LC–MS conditions, as seen in Table II. It was noticed that these compounds had a selective tendency to form ammonium adducts even though the mobile phase did not have an apparent source of the ammonium ions. For example, the cycloethers 25C5 and 30C6 predominantly formed an ammoniated adduct even in the acetic mobile phase system (0.1% acetic acid–MeOH). This indicated that the mobile phase inherently contains trace amounts of ammonium ions (as suggested in reference 18), which have a greater ability to form adducts than the predominant proton ions from the mobile phases. The stable peak area responses of ammoniated 30C6 in five different mobile phases provided further evidence that the instrument system had stable conditions throughout different mobile phase experiments. The formation of several adducts is common in ESI+ process, as opposed to APCI+ (atmospheric pressure chemical ionization), which mainly forms protonated adducts (11). Protonated, ammoniated, and sodiated adducts are common ions seen in LC-ESI-MS, possibly due to the universal presence of trace amount of $NH4^+$ and Na^+ ions. These co-existing multiple ions usually facilitate molecular weight determinations. In APCI, chemical ionization occurs in the vapor state and vaporized molecules form protonated adducts according to their proton affinity (11). Ammonium adducts are less likely as high gas phase temperature may decompose weakly attached ammonium ion. Sodiated adducts are also less likely as sodium ion may not easily vaporize in the gas phase (9, 11). In the ESI, it is assumed that the molecule is charged before final formation of the gas-phase ion, or in other words, the analyte charging has occurred in the ESI charged droplet solution (11). Thus the ESI gas phase ions are formed in more mild conditions than APCI. The gas phase adduct formation may depend on multiple parameters related to solution, instrument, and compound properties. Here we focus on compound structure and its effect on adduct formation.

The strong response of the ammonium adducts seen in 25C5 and 30C6 can be related to the size-fit-ion-in-the-hole complexation effect that had been reported by Tsuda and Oshima for crown ethers (22) in solution. A similar crown ether complexation effect was also reported for sodium ion; while the 15-membered crown ether preferentially formed the sodiated adduct, the 18-membered crown ether preferentially formed the ammonium adduct. Sodium ion complexation with polyoxy compounds in the solution and gas phases are well documented (9, 23). The ammoniated adduct of Irg2 could be related to its poly-functional group and stereotic configuration. The carboxylic side chain can "curve up" with phenyl hydroxyl group, fitting the ammonium ion in between them. Ammonium ions

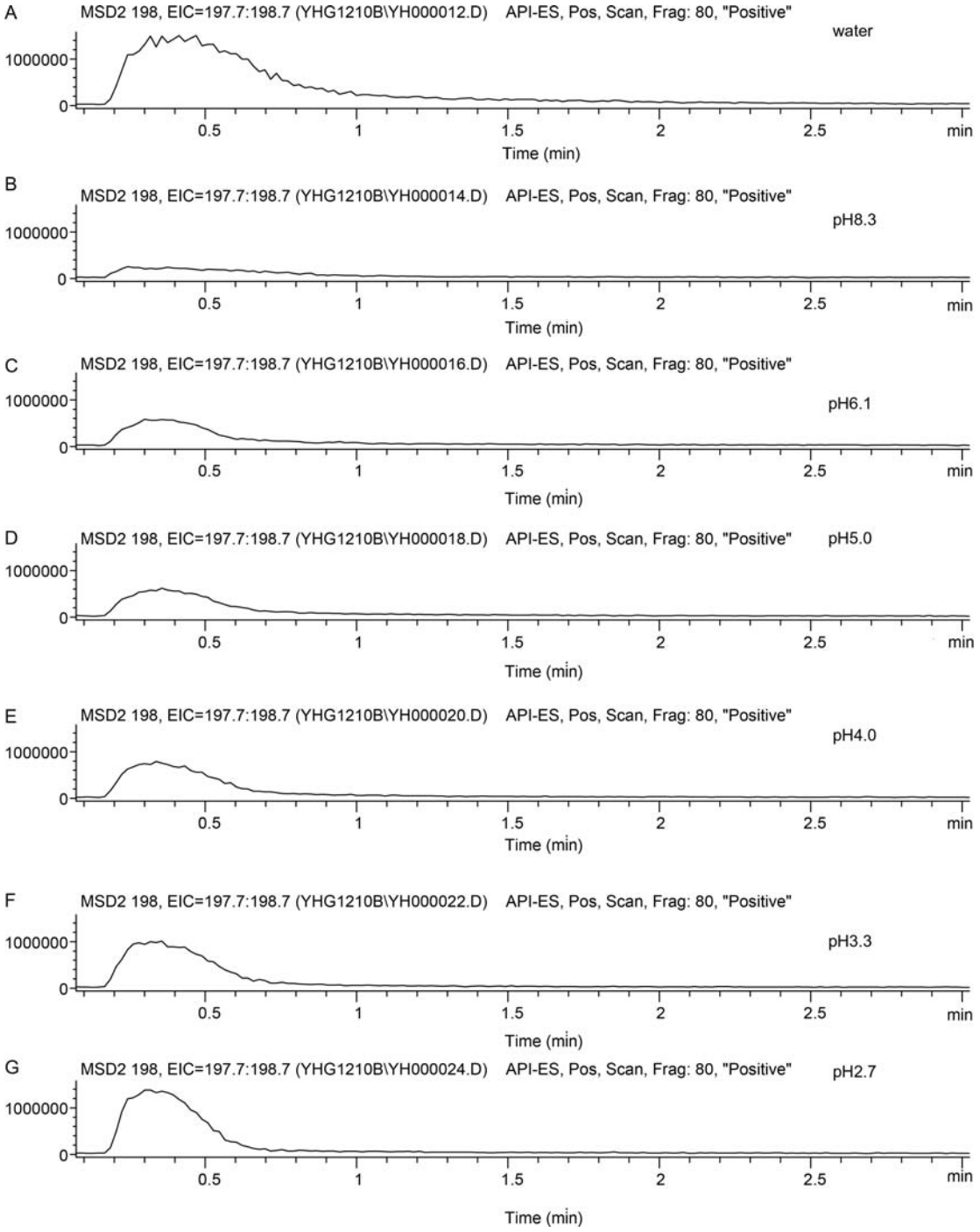


Figure 5. Extract ion response profiles from flow injection analysis of 1 μg/mL (ppm) BA in 1:1 MeOH/varied aqueous solutions: (A) water; (B) pH8.3; (C) pH6.1; (D) pH5.0; (E) pH4.0 buffer solutions; (F) pH3.3 0.1% HAc; (G) pH 2.7 0.1% formic acid. Isocratic mobile phase conditions with 1:1 MeOH/varied aqueous solutions corresponding to sample matrices were used.

were known to solvate polyfunctional compounds to give ammoniated ions in the gas phase, particularly polyoxy compounds (24–25). The ammonium adduct responses did not change significantly among the various mobile phases as seen in Table II, and similar observation was also made by Kamel et al. (9). It is possible that these compounds have a strong ammonium ion binding affinity, and that ammonium ions as a solvent or system impurity is sufficient for adduct formation, as increasing the solution's ammonium ion concentration did not produce significantly more new ammonium adducts.

Negative ions

Acidic extractables such as acids and phenols can form negative ions readily in ESI. The negative ion profiles in varied pH mobile phases are shown in Figure 3 and peak intensity data

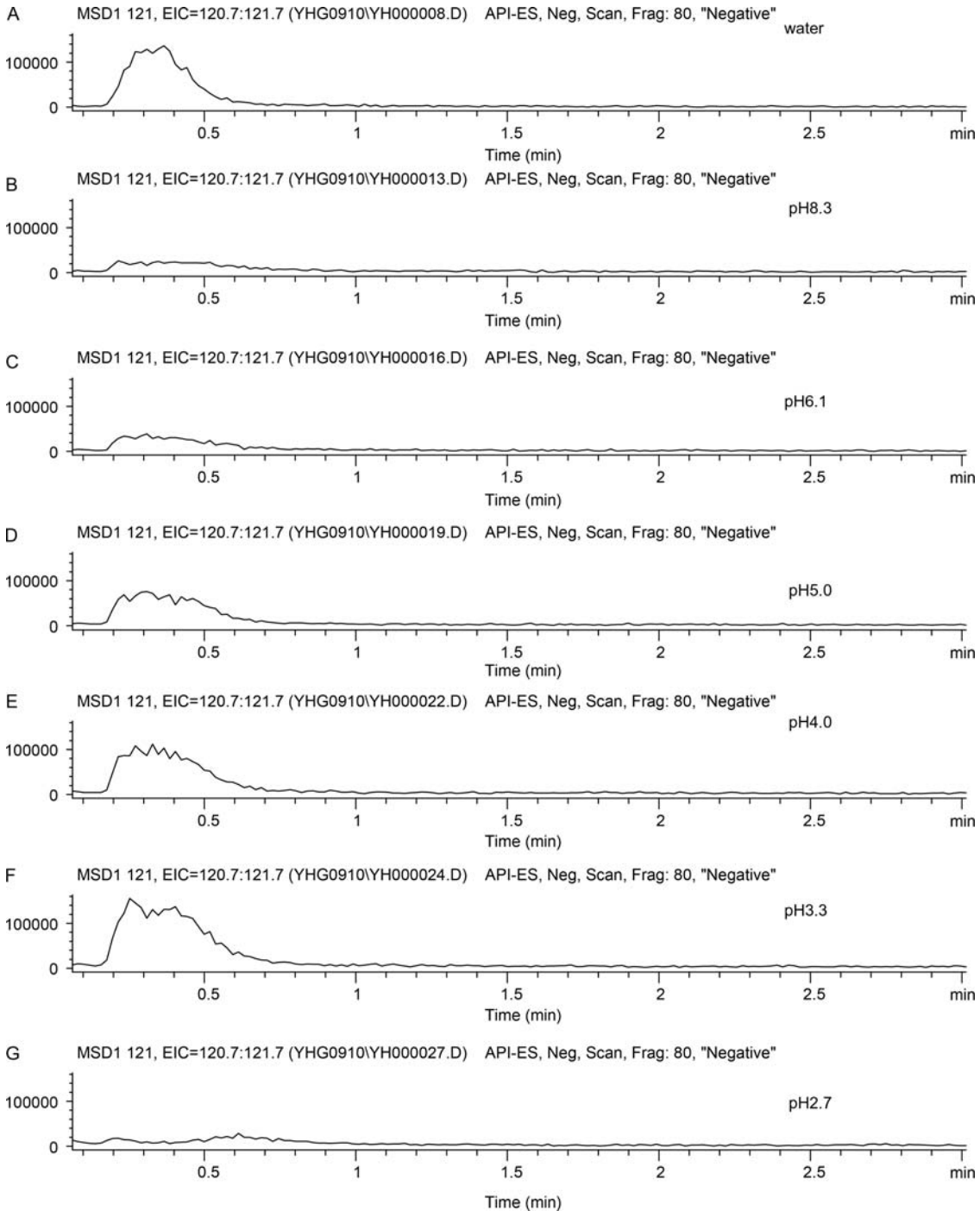


Figure 6. Extracted ion response profiles from flow injection analysis of 500 ng/mL (ppb) DBA in 1:1 MeOH mixtures with various aqueous solutions including (A) water; (B) pH8.3; (C) pH6.1; (D) pH5.0; (E) pH4.0 buffer solutions; (F) pH3.3 0.1% HAc; (G) pH 2.7 0.1% formic acid. The peak intensity of the M-H+ adduct increased with decreasing solution pH.

are listed in Table III. It is noticed that negative ion responses did not decrease with lower pH mobile phases. Actually, for benzoic acid (BA) and 2-ethylhexanoic acid (EHA), the corresponding negative ion responses increased quickly with decreased mobile phase pH (from 8.1 to pH 3.3). Because the retention times of these acids were different as a function of mobile phase pH, FIA analysis of the acids in 1:1 methanol/aqueous solutions with pH ranging from 2.7 to 8.3 were also performed; the results for 1 ppm BA are shown in Figure 6. The negative ion responses for BA increased consistently from pH 8.3 to 3.3 solutions at constant organic content, indicating that weak acids facilitated the formation of ESI negative ions. This relationship between mobile phase pH and analyte response in ESI was observed by other research groups and is

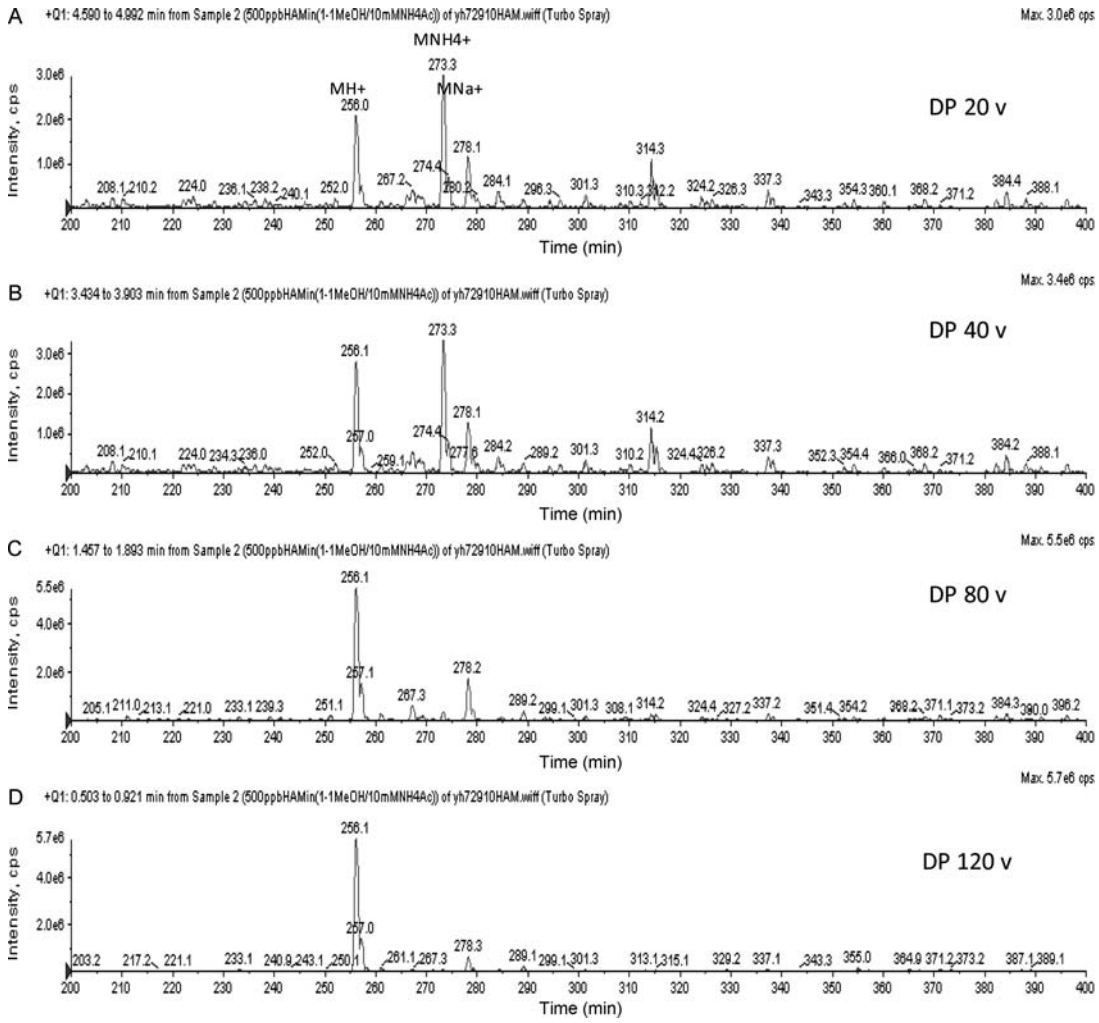


Figure 7. Positive ion mass spectra obtained from infusion of 500 ppb HAM in 1:1 MeOH/10 mM NH4Ac solution at 10 μL/min as a function of decluster potential (DP): (A) 20 v; (B) 40 v; (C) 80 v; (D) 120 v. All spectra represent average of at least 30 scans.

termed as wrong-way-around (9, 11, 26). Two reasons were given by Dalton et al. (26) to account for the wrong-way-around phenomena in negative ESI mode. The electrochemical reaction in the ESI capillary tip could be facilitated is a weakly acidic environment (versus a neutral pH solution). Near the ESI tip, weak acids could provide protons that facilitate the production of excess negative charge by reducing the number of protons to hydrogen gas. These excess charges likely accumulate to a greater extent on the surface of ESI droplets, increasing local pH value and promoting deprotonation of the analytes. Furthermore, acetic acid was chosen as the appropriate acid due to its relatively higher gas phase proton affinity versus some other acids like formic acid and propionic acid.

When formic acid was used in the mobile phase, severe signal suppression resulted, as demonstrated in Figure 6. As strong solution acidity would decrease the pH gradient within the spray droplet, the low gas phase proton affinity of the weakly acidic formic acid retarded the deprotonation process, resulting in a dramatic signal decrease in the negative ion intensities.

Similar to the positive ion mode, deprotonated BA in methanol–water only exhibited high signals similar to pH 3.3 acetic acid solution; this could be due to the decrease of droplet acidity via ESI tip electrochemical reactions and less electrolytes competing for gas phase ionization. When no buffer exists on the droplets, pH could be much higher than neutral in droplet surface environment.

The high responses for the investigated acids in weak acidic mobile phases like acetic acid can be attributed to the previously noted solution effects that occur during ESI process. These effects, when combined with the response benefits associated with the longer retention times in the acidic mobile phases, makes buffers containing acetic acid the preferred choice for negative ESI.

Comprehensive consideration of mobile phase effects

Considering the results for the positive and negative ions experiments, a weakly acidic mobile phase containing some ammonium ion improves sensitivity in both detection modes. Further experiments were performed to more finely tune

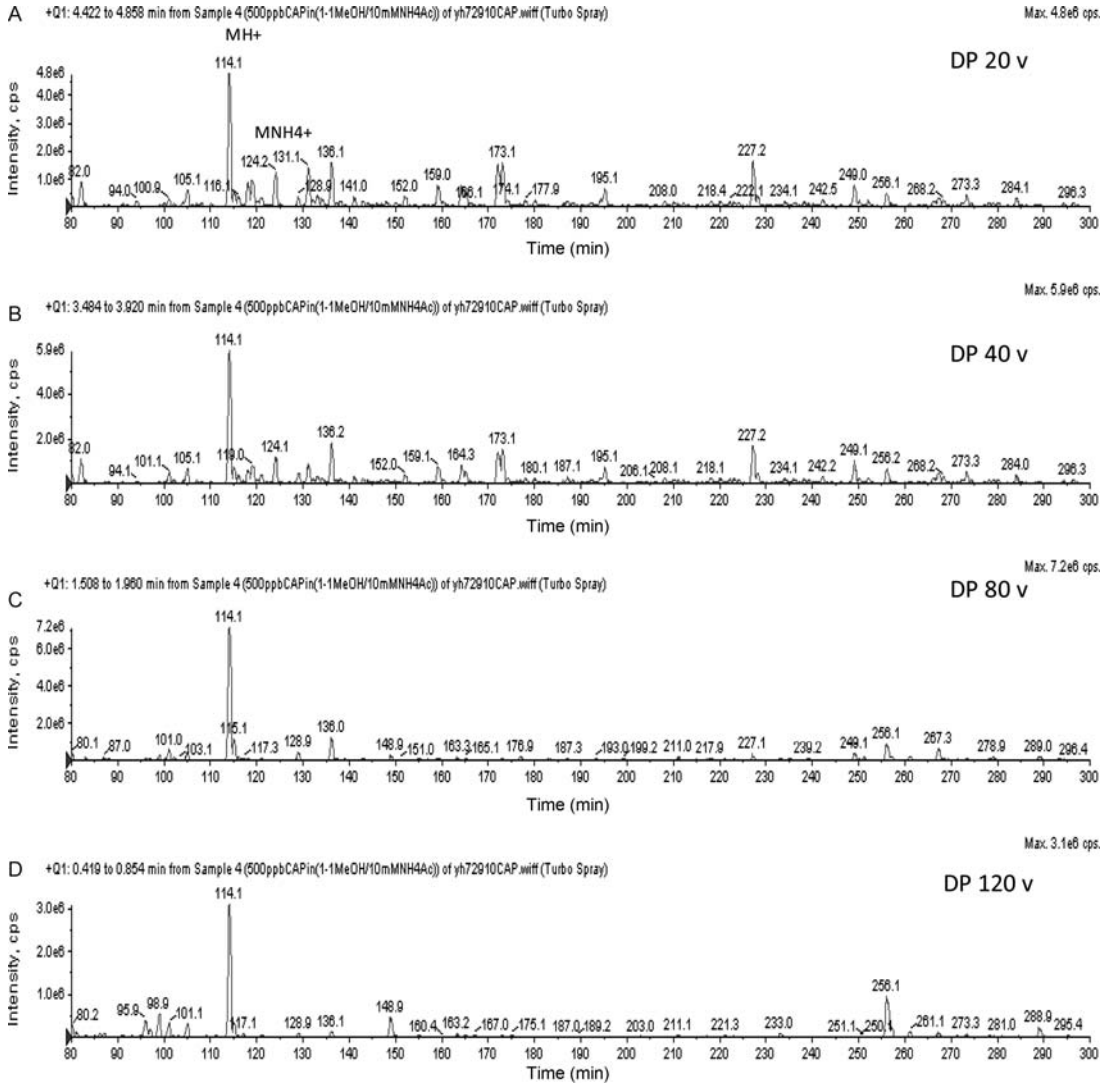


Figure 8. Positive ion mass spectra obtained from infusion of 500 ppb CAP with a 1:1 MeOH/10 mM NH4Ac solution at 10 μL/min as a function of decluster potential (DP): (A) 20 v; (B) 40 v; (C) 80 v; (D) 120 v. All spectra represent average of at least 30 scans.

mobile phase composition, specifically examining four mobile phase systems with aqueous components having pH values of 4.5, 3.6, 3.1, and 3.3 and containing varying amounts of acetic acid, ammonium acetate, and formic acid. The results of these experiments are shown in Figure 9 and 10. Severe negative ion signal suppression across all target compounds was seen using formic acid, even at levels as low as 0.01%. However, certain basic compounds, such as CAP and DBA, had elevated positive ion responses with the 0.01% formic acid mobile phase system, possibly due to its relatively low electrolyte strength. Even compared with 0.1% formic acid (pH 2.7, Figure 2E), 0.01% formic acid mobile phases at a higher pH (pH 3.3) provided better responses for CAP and DBA. All things considered, however, formic acid is not recommended for extractables screening LC–MS due to the poor negative ion sensitivities.

When mobile phases with 0.1% acetic acid in 10 mM ammonium acetate were examined, negative ion signals for BA and EHA and the positive ion signal of CAP was reduced at the higher pH values (for example pH 4.5 versus either 3.6 or 3.1). While the peak responses for the other target analytes were not adversely affected by the higher pH, the pH 4.5 mobile phase produces sub-optimal performance and therefore is not recommended. Although mobile phases at pH 3.6 and pH 3.1 produced comparable responses in positive ion mode, the latter mobile phase produced recognizably lower responses in negative mode for most target compounds. This was the case because high acid concentrations (0.5%) were required to make the pH 3.1 mobile phase that also contained 1 mM ammonium acetate. The reduction of negative ion responses by high acidity mobile phase components modifier was demonstrated by Dolton et al. (26). These researchers performed post-column infusion experiments and suggested that high acidity (greater than 1 mM, 0.007%) could reduce negative ion intensities by reducing the spray droplet surface pH gradient, making deprotonation more difficulty.

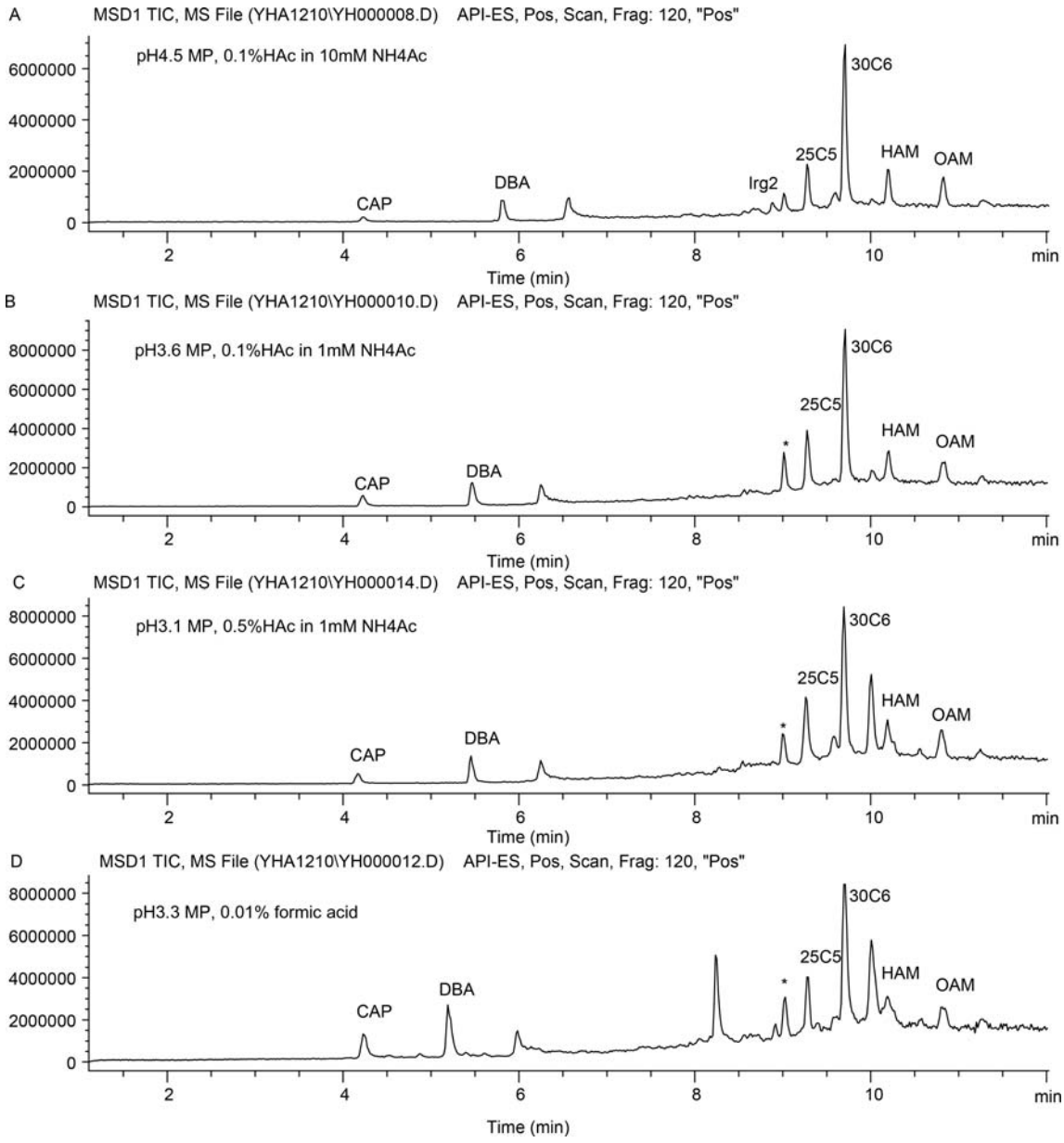


Figure 9. Positive ion ESI LC–MS analysis of the standard mixture using conditions in Table I with varied aqueous mobile phases: (A) pH4.5 0.1%HAc in 10 mM NH4Ac; (B) pH3.6 0.1% HAc in 1mM NH4Ac; (C) pH3.1 0.5% HAc in 1 mM NH4Ac; (D) pH3.3 0.01% formic acid. * Irg2 co-eluted with impurity peaks.

Based on the experiments performed in this study, it is concluded that a pH 3.6 (0.1% HAc in 1 mM NH4Ac) mobile phase system should be used to achieve the best overall LC–MS response for chemically diverse extractables. With the moderate 0.1% HAc concentration, negative ions exhibited optimal responses while positive ions also showed good responses for the different types of extractables examined. Furthermore it is suggested that the weak acetic acid with ammonium acetate mobile phases could be appropriate in other screening-type of applications.

Conclusion

A LC–UV–MS methodology utilizing a pH 3.6 mobile phase system containing 0.1% acetic acid and 1 mM ammonium acetate is proposed as a means of optimizing detectability for the chemically diverse set of chemical compounds that are encountered in extractables screening. This acidic aqueous mobile phase produces optimal responses for negative ion-producing compounds due to the combined effects of acid-promoted electrochemical reaction and favored acetate ion gas phase basicity. This mobile phase system also produces overall favorable responses for positive ion-producing compounds. Although more acidic mobile phases might increase the responses for some basic targets (such as CAP and DBA), they also caused reduced responses for very weak basic compounds such as amides due to lack of ammonium ion effect. Ammonium ions were identified as important mobile phase components, improving the response of protonated adducts of

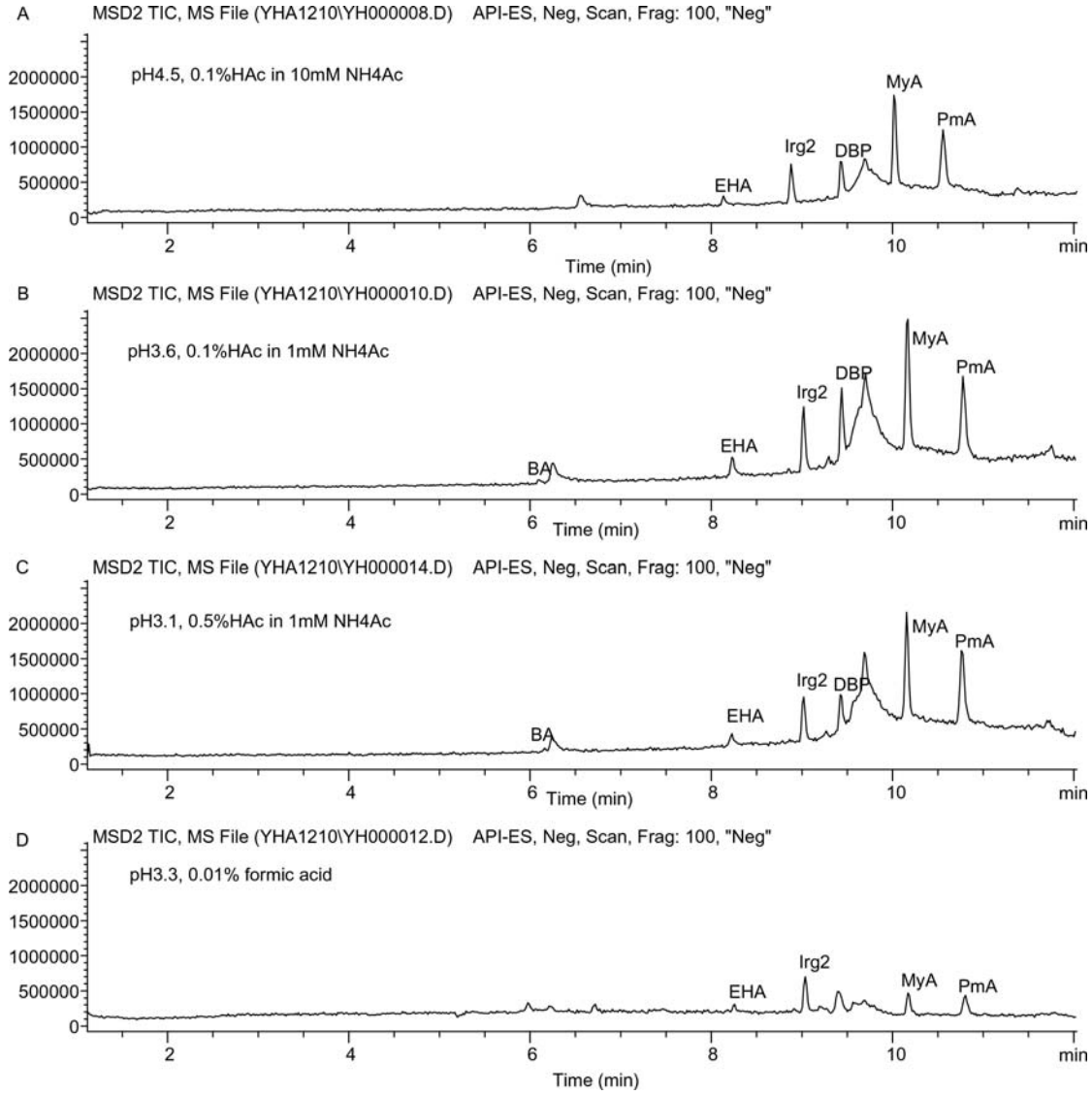


Figure 10. Negative ion ESI LC–MS analysis the standard mixture using conditions in Table I with varied aqueous mobile phases: (A) pH4.5 0.1%HAc in 10 mM NH4Ac; (B) pH3.6 0.1% HAc in 1mM NH4Ac; (C) pH3.1 0.5% HAc in 1 mM NH4Ac; (D) pH3.3 0.01% formic acid.

very weak basic compounds like amides via ammonium adduct conversion. With further study, the general recommendation of this study may also be applicable to other types of multi-component analyses such as those encountered in environmental, food, and pesticide studies.

References

1. Gill, M.; Garber, M.J.; Hua, Y.; Jenke, D.R. Development and validation of an HPLC/MS/MS method for quantitating *bis*-(2,2,6,6-tetramethyl-4-piperidinyl) sebacate (Tinuvin 770) and a related substance in aqueous extracts of plastic materials. *Journal of Chromatographic Science* **2010**, *48*(3), 200–207.
2. Jenke, D.R.; Story, J.; Lalani, R. Extractables/leachables from plastic tubing used in product manufacturing. *International Journal of Pharmaceutics* **2006**, *315*(1,2), 75–92.
3. Yu, X.; Zdravkovic, S.; Wood, D.; Li, C.; Cheng, Y.; Ding, Y.X. A new approach to threshold evaluation and quantitation of unknown extractables and leachables using HPLC/CAD. *Drug Delivery Technology* **2009**, *9*(3), 50–55.
4. Jenke, D.R.; Swanson, S.; Edgcomb, E.; Couch, T.; Chacko, M.; Garber, M.J., etc. Strategy for assessing the leachables impact of a material change made to a container/closure system. *PDA Journal of Pharmaceutical Science and Technology* **2005**, *59*(6), 360–380.
5. McDonald, J.G.; Cummins, C.L.; Barkley, R.M., Thompson, B.M., Lincoln, H.A.; Identification and quantitation of sorbitol-based nuclear clarifying agents extracted from common laboratory and consumer plasticware made of polypropylene. *Analytical Chemistry* **2008**, *80*(14), 5532–5541.
6. Story, J.; Gill, M.; Liu, N.; Hua, Y.; Jenke, Y.D.R. Generation of the extractables profile of an elastomeric materials and investigation of the accumulation behavior of target leachables including *Bis*(2,2,6,6-tetramethyl-4-piperidinyl) sebacate (Tinuvin 770) and a related substance. *PDA Journal of Pharmaceutical Science and Technology* **2010**, *62*(4), 101–12.
7. Jenke, D.R.; Poss, M.; Sadain, S.; Story, J.; Smith, W.; Reiber, D.J. Identification of caprolactam oligomers and related compounds in aqueous extracts of Nylon 6. *Applied Polymer Science* **2005**, *95*, 1262–1274.

8. Garcia, M.C.; Hogenboom, A.C.; Zappey, H.; Irth, H. Effect of mobile phase composition on the separation and detection of intact proteins by reversed-phase liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A* **2002**, *957*, 187–199.
9. Kamel, A.M.; Brown, P.R.; Munson, B. Effects of mobile-phase additives, solution pH, ionization constant, and analyte concentration of the sensitivities and electrospray ionization mass spectra of nucleoside antiviral agents. *Analytical Chemistry* **1999**, *71*(24), 5481–5492.
10. Saint-Marcoux, F.; Lachatre, G.; Marquet, P. Evaluation of an improved general unknown screening procedure using liquid-chromatography-electrospray-mass spectrometry by comparison with gas chromatography and high-performance liquid-chromatography-diode array detection. *Journal of the American Society for Mass Spectrometry* **2003**, *14*, 14–22.
11. Thurman, E.M.; Ferrer, L.; Barcelo, D. Choosing between atmospheric pressure chemical ionization and electrospray ionization interfaces for the HPLC/MS analysis of pesticides. *Analytical Chemistry* **2001**, *73*(22), 5441–5449.
12. Kebarl, P.; Verkerk, U.H. Electrospray: From ions in solution to ions in the gas phase, what we know now. *Mass Spectrometry Review* **2009**, *28*, 898–917.
13. Ikonomou, M.G.; Blades, A.T.; Kebarle, P. Investigations of the electrospray interface for liquid chromatography/mass spectrometry. *Analytical Chemistry* **1990**, *62*(9), 957–967.
14. Ehrmann, B.M.; Henriksen, T.; Cech, N.B. Relative importance of basicity in the gas phase and solution for determining selectivity in electrospray ionization mass spectrometry. *Journal of the American Society for Mass Spectrometry* **2008**, *19*, 719–728.
15. NIST. **2010**) NIST Webbook. http://webbook.nist.gov/chemistry. Accessed February 7, 2012.
16. Ikonomou, M.G.; Blades, A.T.; Kebarle, P. Electrospray-ion spray: A comparison of mechanisms and performance. *Analytical Chemistry* **1991**, *63*(18), 1989–1998.
17. Draper, W.M.; Xu, D.; Perera, S.K. Electrolyte-induuced ionization suppression and microcystin toxins: Ammonium formate suppresses sodium replacement ions and enhances protiated and ammoniated ions for improved specificity in quantitative LC-MS-MS. *Analytical Chemistry* **2009**, *81*(10), 4153–4160.
18. Tang, L.; Kebarle, P. Dependence of ion intensity in electrospray mass spectrometry on the concentration of analytes in the electrosprayed solution. *Analytical Chemistry* **1993**, *65*(24), 3654–3668.
19. Mansoor, B.A.; Volmer, D.A.; Boyd, R.K. "Wring-way-round" electrospray ionization of amino acids. *Rapid Communications in Mass Spectrometry* **1997**, *11*, 1120–1130.
20. Kelly, M.A.; Vestling, M.M.; Fenselau, C.C.; Smith, P.B. Electrospray analysis of proteins: A comparison of positive-ion and negative-ion mass spectra at high and low pH. *Organic Mass Spectrometry* **1992**, *27*(10), 1143–1147.
21. Wang, G.; Cole, R.B. Disparity between solution-phase equilibria and charge state distributions in positive-ion electrospray mass spectrometry. *Organic Mass Spectrometry* **1994**, *29*(8), 419–427.
22. Tsuda, A.; Oshima, T. Effects of host-guest recognition on kinetics of Diels-Alder reaction of Quinocrown ethers with cyclopentadiene. *Journal of Organic Chemistry* **2002**, *67*(4), 1282–1289.
23. Wu, J.; Polce, M.J.; Wesdemiotis, C.J. Unimolecular chemistry of Li+ and Na+-coordinated polyglycol radicals, a new class of distonic radical cations. *American Chemical Society* **2000**, *122*(51), 12786–12794.
24. Westmore, J.B.; Alauddin, JM.M. Ammonia chemical ionization mass spectrometry. *Mass Spectrometry Reviews* **1986**, *5*(4), 381–465.
25. Rudewicz, P.; Munson, B. Effect of ammonia partial pressure on the sensitivities for oxygenated compounds in ammonia chemical ionization mass spectrometry. *Analytical Chemistry* **1986**, *58*(14), 2903–2907.
26. Wu, Z.; Gao, W.; Phelps, M.A.; Wu, D.; Miller, D.D.; Dalton, J.T. Favorable effects of weak acids on negative-ion electrospray ionization mass spectrometry. *Analytical Chemistry* **2004**, *76*(3), 839–847.