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RESEARCH ARTICLE

Acetonitrile Ion Suppression in Atmospheric Pressure Ionization Mass Spectrometry

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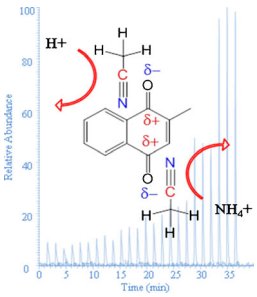

Abstract. Efforts to analyze trace levels of cyclic peroxides by liquid chromatography/mass spectrometry gave evidence that acetonitrile suppressed ion formation. Further investigations extended this discovery to ketones, linear peroxides, esters, and possibly many other types of compounds, including triazole and menadione. Direct ionization suppression caused by acetonitrile was observed for multiple adduct types in both electrospray ionization and atmospheric pressure chemical ionization. The addition of only 2% acetonitrile significantly decreased the sensitivity of analyte response. Efforts to identify the mechanism were made using various nitriles. The ion suppression was reduced by substitution of an acetonitrile hydrogen with an electron-withdrawing group, but was exacerbated by electron-donating or steric groups adjacent to the nitrile. Although current theory does not explain this phenomenon, we propose that polar interactions between the various functionalities and the nitrile may be forming neutral aggregates that manifest as ionization suppression.

Keywords: Acetonitrile ion suppression, Peroxide detection, Menadione, TATP, Energetic materials, Gas phase reactions

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Introduction

Ionization suppression caused by undetected or unknown impurities, contaminants, or solvents has been an ongoing issue for mass spectrometry (MS) users. Whether the issue is caused by one of the numerous possible suppression factors outlined in the literature [1–3] or from reaction (either gas or liquid phase) of the analyte with the matrix [4], these effects compromise the ability to detect the analyte [1–3]. Furthermore, these problems are frequently very difficult to recognize [5]; ion suppression may easily be misinterpreted by the absence of an unknown component that significantly enhances ionization [6]. Efforts to minimize these effects have been extensive for electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), although APCI typically experiences fewer of these issues [2, 7]. Usually co-eluting background interference from matrix components are the most significant problem and frequently can be addressed by changing the liquid chromatography (LC) conditions to separate undetected suppressors from the analyte [5]. Solvent suppression, either from aqueous mobile phase modifiers, pH adjustment, or organic solvent selection is typically identified in the initial analysis for the compound of interest.

Addition of some degree of organic solvent is known to improve ionization in atmospheric pressure ionization (API) sources by improving the volatility of the solution. The organic modification can disrupt surface tension of droplets and generally assists in the droplet evaporation process [8–10]. The two most abundantly used organic solvents in reverse-phase liquid chromatography (RPLC) are methanol (MeOH) and acetonitrile (ACN) [2, 8, 11, 12]. They have low molecular mass, low reactivity, low UV cutoffs (<210 nm), similar dielectric constants, low surface tension, and good solvent strength for RPLC. The ability of ACN to inertly solvate many nonpolar analytes makes it a common first choice for LC/MS analysis. Methanol may be preferred if a more polar or protic solvent is required, or if excessive solvent expense is an issue [13]. However, if chromatographic conditions are not favorable in MeOH, better ionization may be compromised for better peak shape using ACN. In most cases, initial work will show which

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solvent provides a better MS signal or solvation, and LC development will focus on that solvent.

There are a few previous reports of ionization suppression by ACN. For example, Vieno et al., observing the phenomena with high levels of ACN in the mobile phase, contended that nonpolar matrix constituents eluting at the end of the gradient were causing the suppression [5]. Examining BAY 11-7082, Hewavitharana and Shaw accounted for the correlation between increased ACN concentration and decreased $[M + H]^+$ formation asserting that solvent polarity promoted the formation of the dimerized sodium adduct [14]. Duderstadt and Fischer showed some significant signal loss using ACN or acetone versus MeOH for polyalkene compounds of various size and functionality using APCI and atmospheric pressure photoionization (APPI) sources with a gradient chromatography system; the extent of this effect was not fully determined or quantified [11]. Efforts within our lab to analyze trace levels of cyclic peroxides led to the discovery that ACN appears to be suppressing the formation of H^+, NH_4^+, and Na^+ adduct ions. Further investigation into this issue led to the discovery that this effect extended to ketones, linear peroxides, esters, triazole and menadione. Acetonitrile, present at very low solvent concentrations, caused direct ion suppression for multiple adduct types in both ESI and APCI.

Materials and Methods

Chemicals and Reagents

Caution The sensitive organic peroxides mentioned below are powerful explosives. Take all necessary precautions when working with these compounds

General use water, acetonitrile, methanol (all Optima HPLC grade), ammonium acetate (NH_4OAc) (HPLC grade), methyl ethyl ketone and acetone (ACS grade) were from Fisher Chemical (Fair Lawn, NJ, USA). Fluka Analytical LC/MS Ultra CHROMASOLV acetonitrile and bromoacetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). A 1 L solution of aqueous 10 mM NH_4OAc was prepared at neutral pH unless otherwise stated. Hexamethylenetetramine (hexamine), pivalonitrile (trimethylacetonitrile), cyanamide, tetrabutyl ammonium hydroxide (TBAH), cyclopentanone, and cyclohexanone were purchased from Acros Organics (Morris Plains, NJ, USA). 4,4′-Bis(dimethylamino)benzophenone, commonly called Michler's ketone, was supplied by Alfa Aesar (Ward Hill, MA, USA). Hydrogen peroxide (HP, 50%) was purchased from Univar (Redmond, WA, USA). Menadione and diphenyl isophthalate were obtained from MP Biomedical (Solon, OH, USA). Hexamethylene triperoxide diamine (HMTD) was produced in-house by standard methods reported in previous work [15]. Triacetone triperoxide was synthesized according to the method previously published by our group [16]. Methyl ethyl ketone (MEK) peroxides (MEKPs) and MEK/acetone peroxides (MEK/AP) were produced by the addition of equimolar parts of hydrogen peroxide (50% solution), MEK (or 50/50 MEK/acetone) and sulfuric acid. The non-aqueous layer was pipetted into a clean test tube and washed with water. The organic layer (mixture of various MEKPs or MEK/APs) was pipetted into a tared vial, weighed, and immediately diluted to 50 mg/mL with MeOH. Further dilutions were made as needed.

Instrumentation

Using a Thermo Electron LTQ Orbitrap XL and Exactive mass spectrometer equipped with either APCI or ESI interface, ions were generated and introduced into the ion transfer tube set between 180 to 275 °C (depending on the thermal stability of the compound). Tune conditions for positive ion mode APCI infusion experiments (20 μL/min flow) were as follows: discharge current, 5000 μA; N_2 sheath gas, 20 arbitrary units (AU); N_2 auxiliary gas, 10 AU; vaporizer temperature 220–250 °C; ion transfer tube, 14 V; tube lens, 55 V; and skimmer offset, 0 V. ESI conditions were as follows: source voltage, 4200 V; N_2 sheath gas, 15 AU; N_2 auxiliary gas, 2 AU; ion transfer tube, 14 V; tube lens, 85 V; and skimmer offset, 0 V. Mass spectrometer source conditions for flow injection analysis (FIA) were optimized for an aqueous liquid flow of 300 μL/min. This included increasing the sheath gas to 40 AU (ESI) or 35 AU (APCI) and auxiliary gas to 20 AU (ESI) or 16 AU (APCI) to provide better desolvation. Minor voltage changes were made at times to improve signal intensity for some compounds. Orbitrap mass resolution was set to 15,000 for FIA and 30,000 for direct infusion with mass calibrations done as needed using Pierce LTQ ESI positive or negative ion calibration solutions provided by ThermoFisher Scientific. Solvent delivery was performed using a Thermo Electron Accela quaternary pump. Sample injections were performed by a CTC Analytics HTS PAL autosampler directly from either Agilent Technologies amber glass LC vials with PTFE septa or from Analytical Sales and Service polypropylene, 2 mL 96-well plates with preslit silicone plate covers. Sample preparation was done directly in the aforementioned vials or plates. Additional sample injections using identical solvent and sample delivery were performed on a Thermo Electron Quantiva triple quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI) source. Conditions for HESI analysis were: positive ion spray voltage 4200 V; sheath gas 40 AU; auxiliary gas 12 AU; sweep gas 1 AU; ion transfer tube 220 °C; and vaporizer temperature was 200 °C. Ion transfer tube and vaporizer temperatures were 325 °C and 333 °C, respectively, for 1,2,4-triazole analysis. Data collection and analysis was performed with Thermo Xcalibur software ver. 2.2, SP 1.48.

TATP Analysis

Using the LC vials, seven solutions of 1000 μL were made at concentrations of ACN/MeOH/aqueous 10 mM NH4OAc of 50/0/50, 40/10/50, 30/20/50, 20/30/50, 10/40/50, 5/45/50, and 0/50/50. All volumes given as a "percent" are by volume. A solution of TATP (20 μL of 4.5 mM in ACN) was placed into each vial (final concentration of 90 μM, neglecting the addition of 2% ACN). Samples were individually infused onto the LTQ Orbitrap. Initial results suggested that the 2% ACN should not

be neglected and a new 4.5 mM standard solution was created using MeOH. An eighth vial was added to include a 2/48/50 solution ratio and 10 μL of standard was added to each vial (neglecting the 1% addition of MeOH). These samples were re-infused and used to develop the FIA system.

Flow Injection Analysis (FIA)

An adequate number of scans (>10) across the peak and minimal mixing with the flow were required for this system. The system was acquiring full scan data (between *m/z* 50 to 400) at approximately 110 scans per min for the LTQ Orbitrap (the slowest scanning of the three instruments used). Assuming the liquid was non-compressible, tubing length (from injector to detector) and particularly the inner diameter (i.d.) were minimized using Poiseuille's law to keep viscosity differences in the sample plug and mobile phase negligible [17]. The system was optimized using a constant flow of 10 mM NH_4OAc in pump channel B at 300 μL/min flow to deliver sample volumes of 20 μL from a 20 μL injection loop to the LC/MS source. Wash solvent was exclusively 80/20 water/MeOH. Path length from injection port to source was approximately 0.5 m using 0.005″ i.d. red PEEK tubing. The auto-sampler was set for manual control, and peak to peak injection times were determined by needle, valve, and port wash cycles. Contact closure triggered by the auto-sampler started sample acquisition. In order to avoid data loss from the slight delay of analysis start compared with the speed at which the first sample reached the source, the first injection of each run was blank water. Each analysis allowed triplicate injections for each solution with approximately 1.5 min between injections. Some solvents or analytes (e.g., diphenyl isophthalate) were not compatible with the 100% aqueous environment of the mobile phase, so conditions were modified to run with a binary mixture of 50/50 10 mM NH_4OAc/MeOH. Extracted ion chromatograms (XIC) were integrated using the Genesis peak detection algorithm in Thermo Xcalibur Qual Browser.

HMTD Analysis

Since HMTD is poorly soluble in MeOH (but very soluble in ACN), to create an ACN-free solution, a dilute solution of HMTD (4.8 mM) in MeOH was made. This solution was almost imperceptibly cloudy, indicative of a very fine suspension. Prior to removing any sample, the standard solution was quickly vortex-mixed. Injections were made with each final concentration of 48 μM (HMTD was fully solvated at this concentration).

MEKP and MEK/AP Analysis

Immediately following MEKP or MEK/AP synthesis, material was pipetted into a tared vial and dissolved in MeOH to a volume of 50 and 10 mg/mL, respectively. Since this synthesis produces multiple compounds, individual standards are not available and concentrations could not be accurately determined. Positive results for impact sensitivity confirmed the presence of the desired materials. Samples were produced at 10 and 1 μg/mL to assure that the observed results were not a concentration dependent effect.

Alternate Nitrile Solvents

Cyanamide (a white powder with aqueous solubility of 850 mg/mL) was dissolved in water to 786 mg/mL (18.7 M and comparable to the density of ACN). Samples were produced in a constant 20% MeOH while 10 mM NH_4OAc volume was altered to accommodate the increasing volume of cyanamide from 0% to 50%. Trimethylacetonitrile and bromoacetonitrile were immiscible in water; therefore, the 10 mM NH_4OAc was replaced with MeOH for these experiments (nitrile/MeOH going from 0/100 to 50/50). This was duplicated with ACN to assure the effect was consistent in a 100% organic environment.

Other Analysis

All other compounds analyzed were directly weighed into glass vials and dissolved in MeOH to produce concentrations of standard solutions necessary to add 10 μL to produce the final concentrations in mixtures. Final sample concentrations were varied as needed for detection. MEK was run at 4.1 mM, menadione at 0.581 mM, and hexamine required only 14.3 nM. Diphenyl isophthalate was run at 3.14 μM and 1,2,4-triazole was analyzed at 72.4 μM. Cyclohexanone analysis was performed at a concentration 1.02 mM for all experiments unless otherwise stated. For aqueous content analysis, MeOH was added to compensate for volume loss when 10 mM NH_4OAc was reduced from 50%; however, for the 80% aqueous run, the organic ratios were limited so the MeOH/ACN ratio was set at 0/20, 5/15, 10/10 (run 3 times to keep analyses consistent), 15/5, 18/2, and 20/0.

Calibration Curves

A solid sample of diphenyl isophthalate was weighed into a glass vial and diluted to 1.26 mM in MeOH. From this solution, five serial 2:1 dilutions were made in MeOH to the concentration of 39.3 μM. A sample of liquid cyclohexanone was weighed into a glass vial and diluted to 81.5 mM in MeOH. From this solution, five serial 2:1 dilutions were made in MeOH to the concentration of 2.55 mM. For each compound, these standards were used to prepare calibration curves using 500 μL 10 mM aqueous NH_4OAc, 100 μL of standard solution, and 400 μL MeOH. Three additional curves were prepared for each compound by replacing a portion of the MeOH with 20 μL, 50 μL, and 100 μL of ACN (e.g., 20 μL ACN with 380 μL MeOH). All samples were analyzed in triplicate using the FIA system on the TSQ Quantiva. Linear regression was performed using Microsoft Excel ver. 14.0.4760.1000 (32-bit).

Results and Discussion

TATP is very soluble in ACN yet not solvated by MeOH at concentrations above 171 mM (38 mg/mL) [18], far above levels being examined in this work. Literature reports the LC/MS analysis of TATP yielding a significant signal for the ammonium adduct $[M + NH_4]^+$ of *m/z* 240 using APCI [19]. Using a solvent of 50/50 (v/v) aqueous 10 mM NH_4OAc and ACN, no signal of TATP or any related adduct could be observed in either APCI or ESI. Since previous work [19] stated that analysis was performed using MeOH, the solvent was changed to 50/50 (v/v) 10 mM NH_4OAc/MeOH. Infusion of this solution into the APCI source immediately yielded a large signal at *m/z* 240.1442. This anomaly was initially believed to be caused by signal enhancement due to the protic nature of MeOH. Keeping the aqueous portion constant (50% 10 mM NH_4OAc), ACN and MeOH ratios were varied. Rather than a linear increase in signal response corresponding to the increase of MeOH, an exponential increase in response was observed for decreasing ACN levels. Even 2% ACN (the neglected standard volume added to 50/50 NH_4OAc/MeOH) suppressed ionization by as much as 50%. As observed by Annesley for MeOH, it was considered that some trace contamination in the ACN lot might be responsible for this effect [13]. Several lots of Fisher ACN and one lot from Fluka were subsequently tested with identical results to our initial observation.

To determine if the suppression effect of ACN was occurring specifically under APCI conditions, the LC/MS system was switched to ESI. Infusion experiments showed that the effect persisted with similar results. In order to effectively quantify these results, a FIA method was developed to measure peak areas of analytes in specific solvent ratios. The required system had to carry the sample to the source interface with minimal mixing of the mobile phase. While this would require a high flow rate, enough scans across the peak had to be obtained for statistical significance. Sample injection volume and flow rate were optimized for this analysis. Samples were analyzed in triplicate, typically in order of decreasing levels of ACN, but were later run in reverse and random order. The phenomenon persisted for TATP through five separate analyses (excluding the initial infusion experiments) over several months (Supporting Information Table 1).

Previous work with the cyclic peroxide HMTD showed very little response in ESI, so analysis was performed in APCI. Since MeOH showed reactivity toward HMTD in the APCI source, ACN was chosen as the solvent for subsequent APCI analyses [4]. With this work still being in an area of active investigation in our lab, HMTD was examined in the same fashion as described for TATP above. With APCI or ESI, the HMTD signal was significantly more intense when no ACN was present. Irrespective of the ion source, HMTD showed as much as 47% signal suppression with as little as 2% ACN present in the solvent (Figures 1 and 2). With the solubility of HMTD being much greater in ACN, this precludes any notion that the compounds analyzed were simply more soluble in MeOH compared with ACN. Hexamine, the starting material for HMTD synthesis, was also analyzed by this method with no suppression by ACN observed (Figure 2).

With two major cyclic peroxides exhibiting the suppression effect of ACN, we examined MEKPs in ACN. While TATP is the favored aqueous-insoluble product of the reaction of acetone and HP, a similar synthetic route using MEK and HP produces a liquid mixture of linear dihydroperoxy peroxides (DHP), hydroxyhydroperoxy peroxides (HHP), dihydroxy peroxides (DH), and cyclic peroxides (CP) containing one, two, three, and four MEK units. Dissolving freshly made MEKP product mixtures in MeOH for subsequent analysis provided a substantial amount of data in one run. While the moderately sized (300 < MW < 400 Da) MEKP products were only minimally

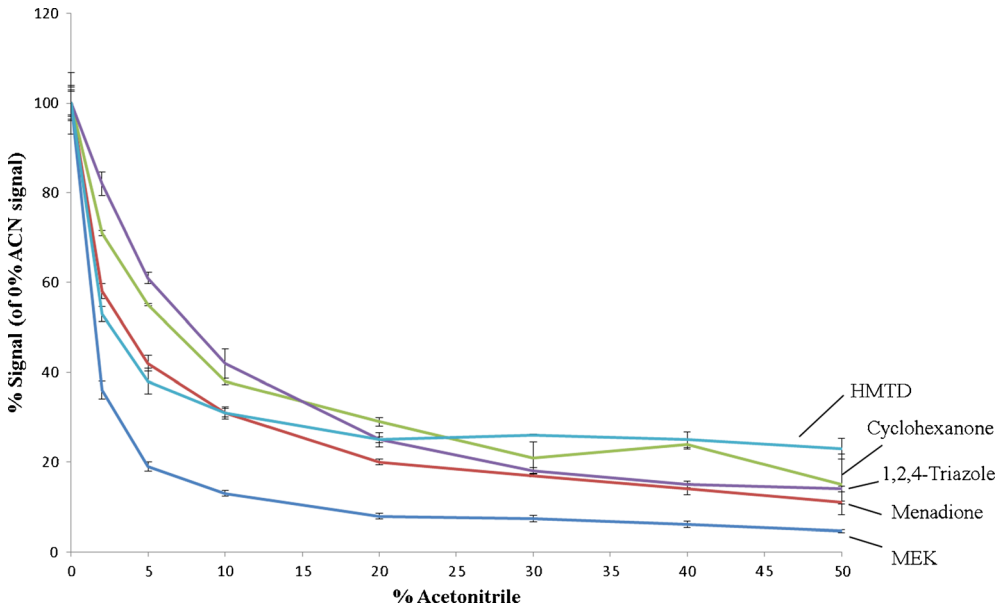


Figure 1. FIA results comparing $[M + H]^+$ relative signal intensity versus %ACN for five compounds in APCI

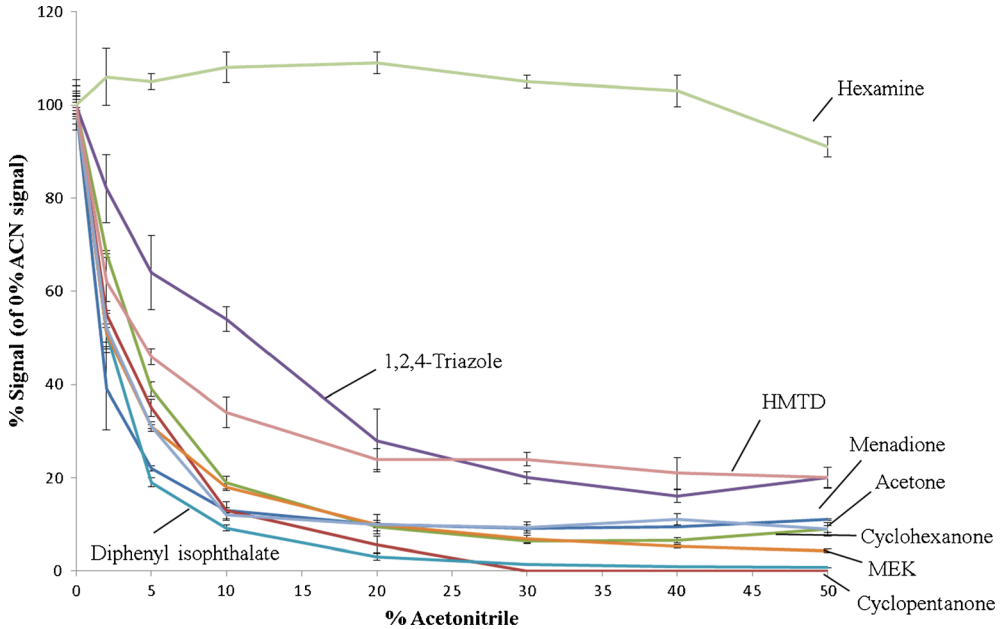


Figure 2. FIA results comparing $[M + H]^+$ relative signal intensity versus %ACN for nine compounds in ESI

affected by the presence of ACN, products over MW 400 Da (DHP4) showed no effect at all. However, some of the smaller (<MW 300 Da) MEKP products were significantly affected. Only data for the 10 µg/mL solution is presented since the signal was completely suppressed for some MEKPs in the 1 µg/mL solution with merely 10% ACN present. All peroxides were detected as adduct ions of either NH_4^+ or Na^+; with the exception of HMTD (only the $[M + H]^+$ observed). The starting materials, MEK and acetone, both showed the ACN suppression effect as well (Figure 2). Analysis of MEKP in the presence of ACN using the TSQ Quantiva with the HESI source (vaporizer temperature at 200 °C) exhibited an even greater suppression effect than had been observed for the ESI source. This was particularly true for the CP3 and DHP3 products that appeared minimally affected under ESI conditions. Figure 3 shows the relative signal loss for the $[M + NH_4]^+$ ions of the peroxide compounds in the ESI source.

To investigate the generality of the ACN ionization suppression effect, other ketones were examined. Significant ACN ion suppression was observed for acetone, cyclohexanone, cyclopentanone, and diphenyl isophthalate (Figure 2), but not for Michler's ketone. Menadione, a vitamin K analog with significant biological roles, has proven to be a difficult molecule to detect by LC/MS [20–22]. The FIA procedure showed that the addition of 2% ACN suppressed menadione ionization by as much as 40% to 60% for APCI and ESI, respectively. All

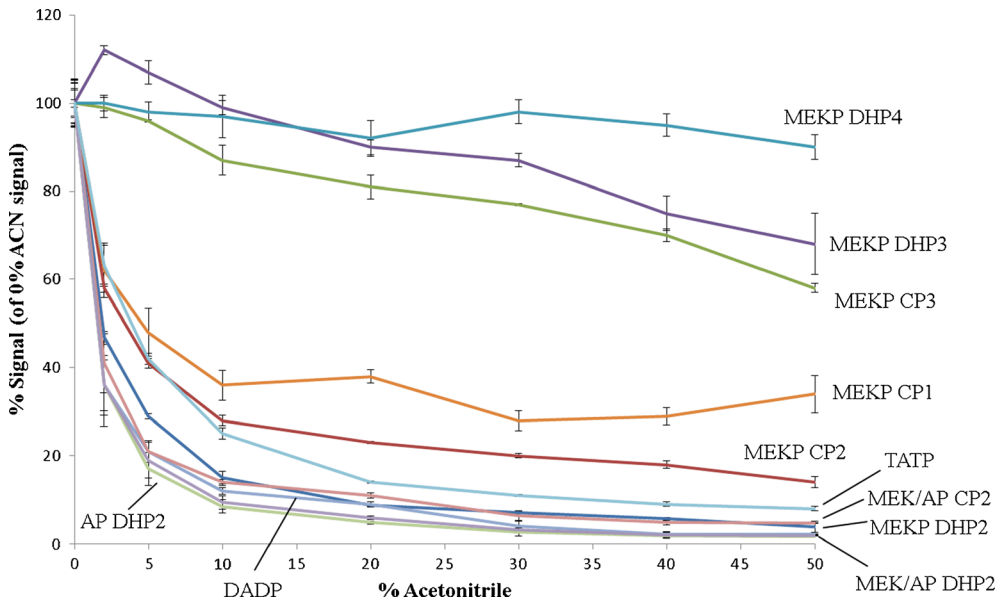


Figure 3. FIA analysis results comparing the $[M + NH_4]^+$ relative signal intensity versus %ACN for 11 peroxide compounds in the ESI source

the ketones, except Michler's, showed both $[M + NH_4]^+$ and $[M + H]^+$ responses affected by ACN with both ESI and APCI. Additional experiments using cyclohexanone and diphenyl isophthalate, with no NH_4OAc added to either the mobile phase or the sample, showed consistent ACN-dependent signal reduction (no $[M + NH_4]^+$ ion was observed for cyclohexanone in APCI under these conditions).

Cyclohexanone was chosen for additional testing since it was readily available, showed a stronger response than the other ketones tested, was safer and more stable than the peroxides, and showed good response for both $[M + H]^+$ and $[M + NH_4]^+$ ions. The signal for cyclohexanone was considerably more intense by APCI, and the concentration had to be lowered from 1.02 mM to 10.2 μM or signal saturation occurred. Most additional experiments were conducted under ESI conditions unless otherwise stated. The standard 50% aqueous NH_4OAc portion was changed to 0%, 5%, 20%, or 80%, and the remaining percentage was made up with varying ACN/MeOH ratios. The signal response was insensitive to the aqueous environment, but highly dependent on the ACN concentration (Supporting Information Figure 1 and Table 1). To further test the effects of the aqueous environment, an acidified aqueous NH_4OAc solution (~pH 3 with 0.1% formic acid) was used in both the sample and the mobile phase, which showed ion suppression was still ACN dependent.

To evaluate the sensitivity effects of ACN ion suppression for the $[M + H]^+$ and $[M + NH_4]^+$ ions, calibration curves were produced and analyzed on the TSQ Quantiva for cyclohexanone and diphenyl isophthalate. Calibration curve slopes (sensitivity) were determined over each compound's dynamic range at four ACN concentrations (0%, 2%, 5%, and 10%) keeping a constant 50% 10 mM NH_4OAc and varying the levels of MeOH. The dynamic range with no acetonitrile present was between 81.5 and 2.55 mM for cyclohexanone and between 1.26 and 0.0393 mM for diphenyl isophthalate. The reduction in sensitivity caused by the ACN addition is expressed as the percent of the slope of each curve to the slope of the curve without ACN. Data for the calibration curves is shown in Table 1 (with 0% ACN added being 100% signal). All correlation coefficients were between 0.974 and 0.999. Single concentration response data (Figure 2 above, Supporting Information Table 1), which shows a consistent decrease in ion response as ACN concentration is increased, is mirrored over the entire calibration curve dynamic range from 0% to 10% ACN concentration.

Table 1. Relative (to 0% ACN) Sensitivities of Cyclohexanone and Diphenyl Isophthalate Using HESI Source

Compound	Ion	%ACN		
		10	5	2
Diphenyl isophthalate	$[M + NH_4]^+$	19	30	58
Diphenyl isophthalate	$[M + H]^+$	22	37	63
Cyclohexanone	$[M + NH_4]^+$	41	52	69
Cyclohexanone	$[M + H]^+$	36	47	66

With the small molecules being used for this study, we considered the ion evaporation model for ESI. Both ACN and MeOH have comparable surface tension and relative permittivity, making them excellent solvents to overcome the Rayleigh charge condition for solution ions to escape into the gas phase [8]. It may be that the ACN is preventing the neutral analyte molecules from forming ions prior to ejection from the charged droplets. However, considering that the ion suppression effect of ACN is observed in APCI as well as ESI, it appears that this phenomenon must be occurring in the gas phase. It may be that certain analytes are emitted from the charged droplets as neutral molecules, which can then undergo gas-phase reactions with other charged reagent molecules similar to APCI. This would suggest that for some molecules, there is a convergence of theories for APCI and ESI, where ultimately gas phase conditions prevail prior to charged ions being detected. If the cause is high volatility, it may explain the reason TATP was affected (since it is known to sublime) [23]. However, this idea falls short when considering HMTD has such a low vapor pressure that it cannot be accurately measured and must be estimated [24].

To explain the source of ion suppression observed for some analytes with ACN, the theory applied in APCI was considered. For the volatilized analyte to be ionized, it must have a higher proton affinity than the reagent molecules [7]. Literature values for the proton affinity (PA) and gas-phase basicity (GPB) data for some of the solvents and analytes used are readily available online (Table 2, Supporting Information) [25]. Both values for ACN (PA 779.2 kJ/mol, GPB 748 kJ/mol) are considerably lower than those of the analytes presented. With PA being defined as the $-\Delta H°(T)$ at temperature (T) for reaction (1), protonation of the analytes (MH^+) should be favorable over ACN protonation (*m/z* 42, $ACNH^+$) [25]. Furthermore, with proton transfer from the analyte to ACN being an endothermic process [12], it might be possible that the heat from the HESI source could allow this endothermic reaction to occur, but this has yet to be clearly demonstrated. Analytes may be within a temperature range that is thermodynamically insignificant since the perceived temperature of the ion/molecule under these conditions can only be estimated. The PA data for the ACN dimer (*m/z* 83, $(ACN)_2H^+$) is unknown, but analogous methyl-substituted imidazole and pyrazole compounds suggest this PA value may be considerably higher (900–960 kJ/mol) than the ACN monomer or the analytes [12]. Although minimal $(ACN)_2H^+$ ion was detected in the presence of NH_4OAc, we did detect high levels (1.1×10^{-7} height counts—comparable to the $[M + H]^+$ of cyclohexanone without ACN present for that analysis) of *m/z* 59 ($ACN\text{-}NH_4^+$), which decreased in parallel with decreasing levels of ACN. This could explain the reason ammonium adduct levels were affected, but it is unclear why the proton adduct would also be suppressed. With no ammonium present, the levels of ACN dimer were significant (6.7×10^{-7} height counts—just under the $[M + H]^+$ of cyclohexanone without ACN present for that analysis). It may be that the dimer or the ammonium adduct of ACN scavenged the positive charge, reducing the formation of

analyte ions. However, this does not explain the reason the ammonium adduct was reduced proportionally to the proton adduct. With the understanding that solvated molecules will increase the proton affinity for the analyte [12], it may be that the analyte-solvent cluster for these compounds increased the proton affinity for ACN and therefore did not form analyte ions (reactions 2 and 3). The charged, intermediate ACN adducts (in brackets) were not detected in any of the analyses, suggesting that this may not be the case.

$$M_{(g)} + H^{+}{}_{(g)} \rightarrow MH^{+}{}_{(g)} \quad (1)$$

$$M_{(g)} + ACN_{(g)} \rightarrow M \cdot ACN_{(g)} + H^{+}{}_{(g)} \rightarrow [M \cdot ACN \cdot H^{+}{}_{(g)}] \rightarrow M(g) + ACNH^{+}{}_{(g)} \quad (2)$$

$$M_{(g)} + ACN_{(g)} \rightarrow M \cdot ACN_{(g)} + NH_4{}^{+}{}_{(g)} \rightarrow [M \cdot ACN \cdot NH_4{}^{+}{}_{(g)}] \rightarrow M(g) + ACNNH_4{}^{+}{}_{(g)} \quad (3)$$

With PA/GPB failing to fully explain the suppression phenomenon, determining the mechanism of ACN ion suppression was attempted by substituting ACN with pivalonitrile (TMACN), cyanamide, or bromoacetonitrile (BrACN). These nitriles were tested against cyclohexanone to determine if they would behave similarly to ACN with regards to ion suppression. Since TMACN and BrACN were immiscible in water, the aqueous portion was replaced with MeOH (also tested against ACN). The electron donating properties of cyanamide were expected to exacerbate the ion suppression, whereas the electron-withdrawing Br on ACN was expected to improve analyte signal. Both cyanamide and BrACN performed as expected as can be seen in Figure 4. However, it should be noted that cyanamide produced multiple, intense ion clusters up to four units with multiple adducts, but none was associated with cyclohexanone. TMACN extensively diminished the analyte signal, consistent with its higher PA (810.9 kJ/mol) compared with other nitriles tested. However, the TMACN proton affinity was still 30 kJ/mol lower than that of cyclohexanone (841 kJ/mol).

Since the majority of molecules in this study contained carbonyl or peroxide groups, 1,2,4-triazole was examined. Although it has a PA of about 100 kJ/mol higher than ACN, it was significantly affected by ACN in all three sources used (APCI, ESI, HESI, Supporting Information Table 1). We initiated a study of other molecules frequently analyzed in our lab. Nitroarenes and nitrate esters examined in negative ion mode MS exhibited no ion suppression with ACN. However, initial indications for nitramines suggest ACN may be inhibiting ionization, and further investigation into this continues. As noted previously, only hexamine, DHP4, and Michler's ketone were completely unaffected by ACN. TBAH, a quaternary ammonium, was tested to determine if ACN could affect a charged species. As expected, the signal for TBAH was not affected. Figure 5 summarizes the species tested, grouping by adducts formed (hydronium and/or ammonium), and the effect of ACN on their ionization.

Nitrile and carbonyl groups have large dipole moments [26] with the electron densities primarily around the nitrogen and oxygen. The electron configuration of nitrile can be arranged to mimic a carbonyl (i.e., they become isosteres) [27]. When polarization occurs with a significant excess of nitrile present (compared with the analyte), a neutral clustering of molecules may form, as shown in Scheme 1. Once clustering occurs, the site of analyte ionization is blocked by the functional group

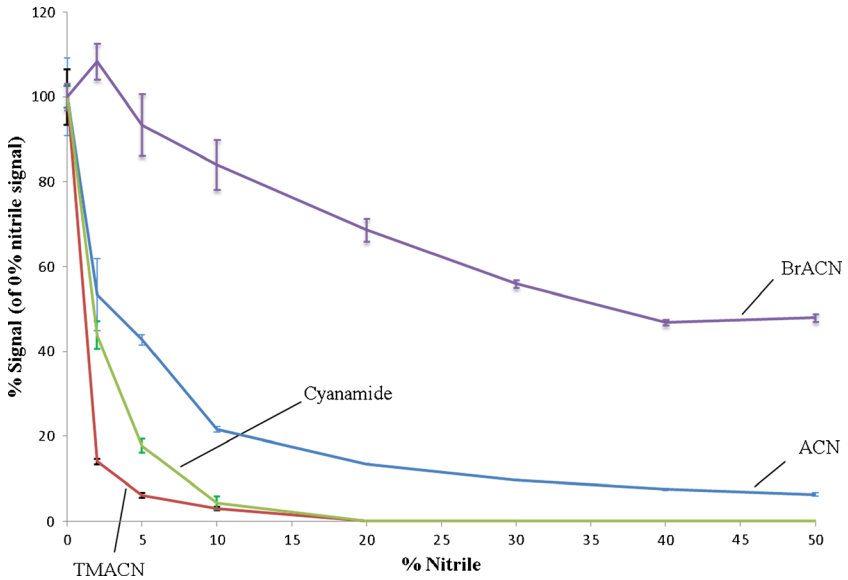


Figure 4. FIA analysis results comparing the cyclohexanone $[M + H]^+$ relative signal intensity versus %nitrile for four different nitrile compounds tested in the ESI source

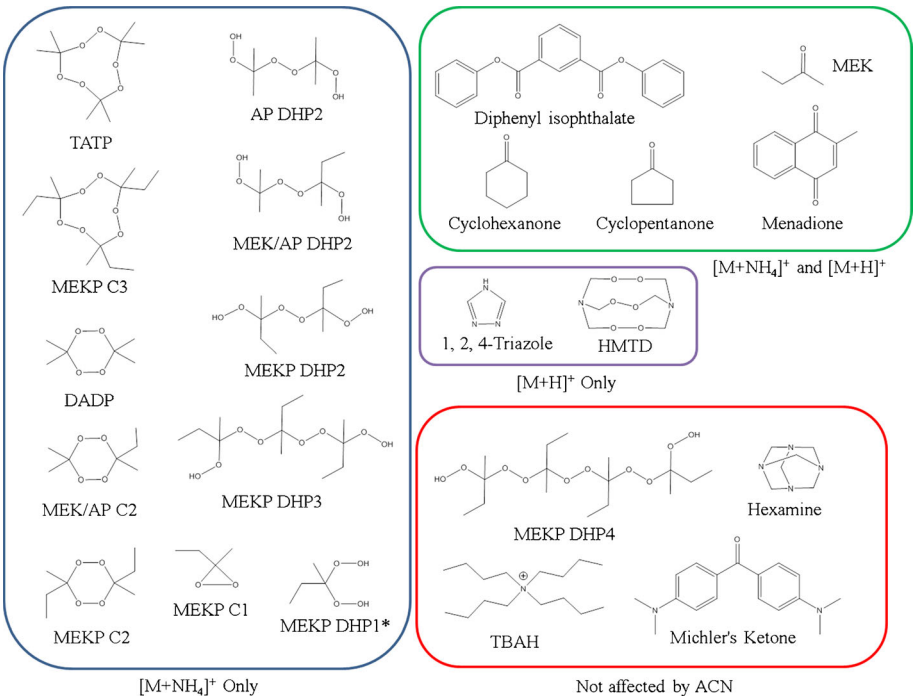
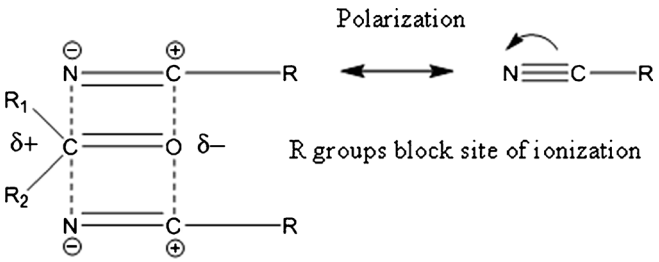


Figure 5. Structures tested for ACN ion suppression (*only one compound produced a sodium adduct)

attached to the nitrile. Furthermore, the excess electrons of the nitrile are not accessible for charge formation while occupied with the carbonyl. With a neutral aggregate formed, the mass spectrometer has no ability to break these clusters as it would with a charged analyte. Formation of this type of aggregate could explain the occurrence of steric, electron-donating, and electron-withdrawing nitriles. Peroxides have small dipole moments in the *trans* configuration but quite large in the *cis* configuration [28]. Cyclic peroxides are forced into a *cis* configuration, thus making them susceptible to acetonitrile suppression. Linear peroxides are free to rotate, though energy input via heat may favor the *cis* configuration. Large linear peroxides would be forced into self-interaction, allowing some *trans* configuration, making them available for ionization. The cyclic MEKP CP3 may have been less affected by ACN than TATP because of the steric interaction of the additional methyl group. Heating may alter the molecular conformation of MEKP CP3 and DPH3 allowing nitrile interaction, which could explain their increased suppression in the HESI source. All data results including comments on analysis can be found in the Supporting Information Table 1 (On-line Resource 1).



Scheme 1. Possible mechanism for neutral aggregate formation with nitriles through polar interaction

Conclusions

With little success we attempted to correlate the ACN suppression effect to ion size and shape, functionality, volatility, gas phase energy, and solvation. This has been rigorously tested in multiple mass spectrometers with different ionization sources. Currently accepted mechanisms for ion formation fail to fully explain the phenomenon. Although the mechanism is still unclear, we have tentatively proposed a polarity aggregation model involving nitriles and carbonyls, peroxides, or other polar molecules that may inhibit ionization. An important objective to this work is alerting the LC/MS community to the significant ion suppression that may be caused by the presence of ACN. Chemical analysis/trace detection of peroxides, ketones, and related compounds would be particularly impacted fields.

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