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A technique for the identification and direct analysis of hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine in metalworking fluids using electrospray-mass spectrometry

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Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine is a widely used biocide in metalworking fluids that resists direct quantification in many analytical methods due to instability. It can be detected in electrospray-mass spectrometry (ES-MS) due to the formation of a charged and relatively stable adduct with the sodium ion. This adduct produces a distinct ion spectrum *via* collision-induced fragmentation, which should promote specific detection of the analyte in complex matrices. ES-MS detection of the analyte added to, or already present in, metalworking fluid samples at μg mL^{-1} levels is demonstrated. Parameters affecting the formation and detection of the sodium adduct, including choice of solvent, alkalinity, and sodium ion level are explored. Linearity of response in flow injection mode is demonstrated.

Introduction

Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine, referred to hereafter as TA, is a widely used biocide that is added to water-based metalworking fluids (MWF) to control populations of microorganisms. Uncontrolled concentrations of this biocide in MWF have been implicated in recent outbreaks of hypersensitivity pneumonitis, possibly because it may induce development of biocide-resistant mycobacteria and subsequent overgrowth of these organisms in MWF sumps even as it eliminates other, competitive microorganism populations.[1,2] The mycobacteria are suspected agents in the induction of pneumonitis.[1–3]

As shown in Fig. 1, TA (**1**) is readily protonated under acidic conditions (which may exist in the immediate vicinity of target microorganisms due to metabolic activity)[4,5] to form a relatively unstable species (**4**), which is believed to decompose through an oxazolidine intermediate (**5**) to ethanolamine (**6**) and formaldehyde (**7**). The latter is presumably the actual antimicrobial agent.[4] To provide optimal treatment of MWF sumps with TA, an analytical method is needed to assess the residual concentration of the compound in MWF. However, there appear to be no methods available for direct measurement of TA. In-house attempts to analyze TA *via* gas chromatography have been unsuccessful due to its thermal lability. In a recent study of MWF, the analyte was measured indirectly by conversion to formaldehyde, which was derivatized to form diacetyl dihydrolutidine and analyzed by high-performance liquid chromatography (HPLC) with diode array detection.[6]

As part of a larger project to identify and quantify MWF components using HPLC with a variety of detectors, including mass spectrometric,[7] the authors have pursued a direct method of analysis of the biocide using electrospray-mass spectrometric detection (ES-MS). The procedure takes advantage of the fact that, as indicated in Fig. 1, TA (**1**) readily forms comparatively stable sodiated adduct monomers (**2**) and dimers (**3**) in certain solvents. These charged species can be analyzed *via* ES-MS. Adduct response has been examined *via* a flow injection technique as a prelude to liquid chromatographic method development. Media which best enhance adduct formation may also be capable of stabilizing the biocide for extended storage periods prior to analysis, and relevant parameters are being evaluated. This report describes our results to date.

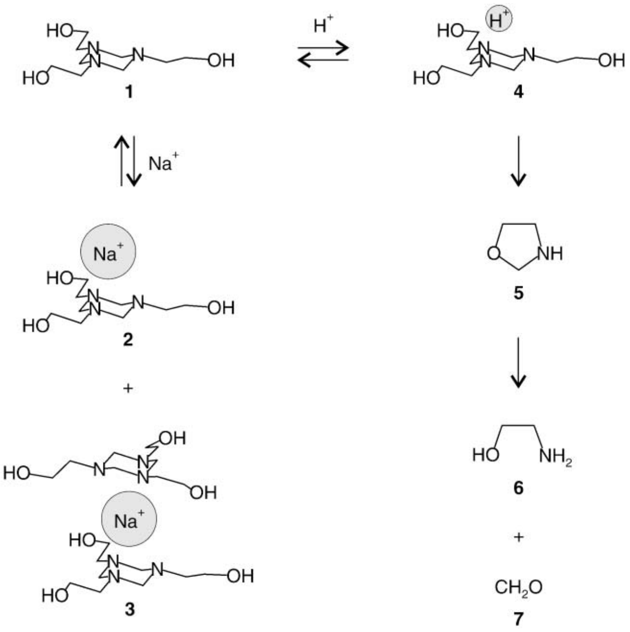

Fig. 1 Assumed reactions of TA (hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine) in presence of hydrogen or sodium ions.

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† Disclaimer: Mention of product name does not constitute endorsement by the Centers for Disease Control and Prevention (CDC).

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Experimental

Compounds

Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine (CAS No. 4719-04-4) was obtained as Bioban™–GK from Angus Chemical Company (Buffalo Grove, IL) (78.5% active agent, 21.5% inert content). *N*,*N*-Dimethyloctylamine (95%), 1-propanol (99.7% anhydrous grade), lithium acetate dihydrate (99.999%), and potassium acetate (99.98%) were obtained from Aldrich Inc. (Milwaukee, WI). Acetonitrile and methanol (both Optima grade), ammonium acetate, acetic acid (HPLC grade), and ammonium hydroxide (Optima grade) were obtained from Fisher Scientific (Pittsburgh, PA). Sodium acetate was Sigma Ultra anhydrous grade from Sigma Inc. (St. Louis, MI). Water was from an in-house Nanopure Ultrapure water system from Barnstead/Thermolyne (Dubuque, IA). The metalworking fluids examined in this report are proprietary and hence are not identified by brand name or otherwise, save for the level of TA quoted from the material safety data sheet (MSDS) for one fluid.

Equipment

Mass spectrometric data was obtained on a Finnigan LCQ Duo equipped with a Spectrasystem P4000 HPLC pump, AS3000 autosampler, and SCM 1000 degasser, all from ThermoFinnigan (Schaumburg, IL). Initially the analyte was monitored with ion lens voltages, desolvating gas flow rates, and other parameters affecting ion signal intensity fixed at a standard default setting (Factory Tune). Once optimal parameter settings for TA detection had been determined, they were saved in tuning files that were used thereafter to provide the most intense ion signals for the analyte. Separate tuning files were generated for low flow (syringe infusion) and high flow (flow injection) conditions (Table 1). Infusion was performed at 25 μL min^{-1} using a syringe pump built into the MS instrument, while flow injection experiments (injection volume 10 μL) used the HPLC pump for solvent delivery at 230 μL min^{-1}. Connective tubing used for flow injection work was polyetheretherketone (0.10 mm id). Serial dilution of samples and standards was performed using EDP-2 motorized pipettes (100 and 1000 μL capacity) from Rainin (Woburn, MA) and glass volumetric flasks from Fisher Scientific. Due to their high viscosity, aliquots of undiluted metalworking fluids were measured using a Gilson Microman positive displacement pipette from Rainin.

Table 1 LCQ-Duo instrument parameters[a]

	Tuning file name		
Parameter	Factory tuning[b]	TA low flow[b]	TA flow injection[c]
Sheath gas[d,e]	20	20	55
Auxiliary gas[d]	0	0	20
Spray voltage	4.50 kV	4.50 kV	5.00 kV
Desolvation capillary voltage	10.00 V	11.00 V	−45.00 V
Tube lens offset	0.00 V	15.00 V	−45.00 V
Multipole 1 offset	−3.00 V	−2.25 V	−2.25 V
Multipole 2 offset	−7.00 V	−5.00 V	−5.00 V
Settings held constant unless noted in text			
Desolvation capillary temperature	200 °C		
Ion lens voltage	−16.00 V		
Multipole RF ramp	400.00 V		
Mode of operation	Microscans per data point	Maximum injection time per microscan/ms	
MS scan	3	50	
MS/MS scan	3	200	
Single ion monitoring	5	200	

[a] All data were collected in positive ion mode using XCalibur Revision 1.2 software. Typical operating pressures were 8.0 × 10^{-1} Torr for the ion lens region and 1.50 × 10^{-5} Torr for the mass analyzer. Collision-induced dissociation and ion energy damping used 99.998% He delivered at 45 psig to a restriction capillary. [b] Used for syringe infusion at 25 μL min^{-1}. [c] Used for flow injection at 230 μL min^{-1}. [d] Gas flow settings are unitless. [e] Minimum flow for sheath gas is 20.

Results and discussion

Detection of sodiated adduct

Detection in ES-MS requires the formation of a charged species. For amines, this is typically accomplished using a mildly acidic environment, which is problematic in the case of TA as noted above. Attempts to detect protonated TA in methanol and methanol–water mixtures produced very weak ion signals for the analyte even with addition of 0.50% NH_4OH (v/v), presumably due to proton donation from the solvent components and rapid degradation. Ammoniated adducts were not observed in these or subsequent experiments. Nonprotic solvents were then investigated in the hope of stabilizing the analyte. In 100% acetonitrile, a weak ion signal was obtained for $[TA+H]^+$ at *m*/*z* 220, but the strongest signals were for the sodiated adduct $[TA+Na]^+$ at *m*/*z* 242 and for the sodiated dimer $[TA_2+Na]^+$ at *m*/*z* 461 (Fig. 2A). Presumably, TA efficiently chelates the sodium ion *via* interaction with the free electron pairs on the ring nitrogens and "sandwiches" the metal

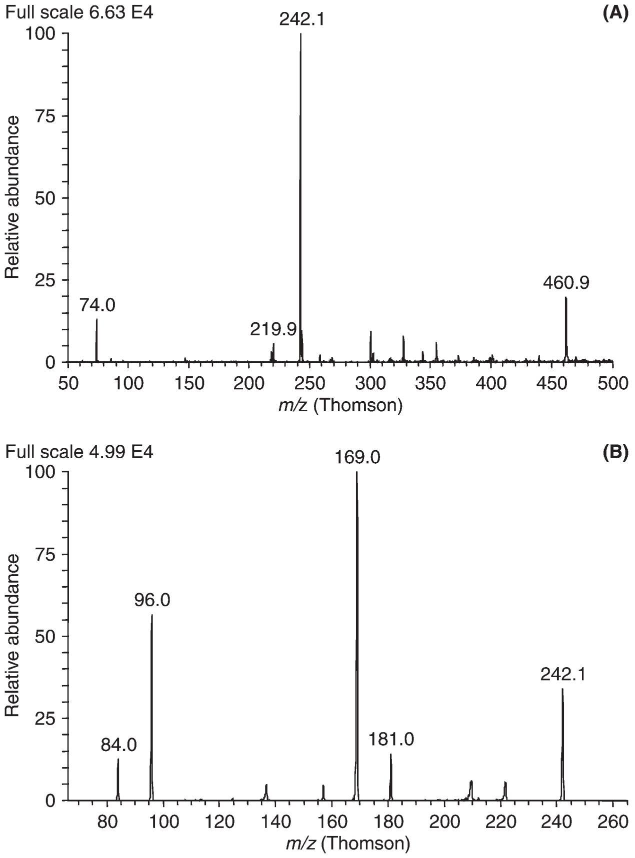

Fig. 2 (A) Mass spectrum of 1.0 μg mL^{-1} TA in 100% acetonitrile, infused at 25 μL min^{-1}. Ions are visible for $[TA+H]^+$ at *m*/*z* 220, $[TA+Na]^+$ at *m*/*z* 242, and $[TA_2+Na]^+$ at *m*/*z* 461. (B) Collision-induced fragmentation of $[TA+Na]^+$ at 27% of full-scale collision energy. Main fragment ions reflect loss of two $[CH_2NCH_2CH_2OH]$ at *m*/*z* 96 and loss of one $[CH_2NCH_2CH_2OH]$ at *m*/*z* 169.

ion between two molecules of TA by the same mechanism [(**2**) and (**3**) in Fig. 1]. Na^+ apparently leached from the glass walls of solvent containers or the volumetric flasks in which samples were prepared. Sodium adduct formation provides a convenient method for detecting TA *via* ES-MS without relying on protonation and attendant loss of analyte.

Collision-induced dissociation was performed on the $[TA+Na]^+$ precursor ion. The resulting fragment ion pattern was as expected, given that TA is a symmetric molecule and all ring nitrogens should share the sodium ion equally. The main fragment ions represent $[CH_2NCH_2CH_2OH+Na]^+$ and $[HOCH_2CH_2NCH_2N(CH_2)CH_2CH_2OH+Na]^+$ (Fig. 2B). Electrospray combined with confirmatory fragmentation should thus, in theory, allow highly specific and sensitive detection of TA, even in complex sample matrices.

In unrelated experiments, it had been noted that 1-propanol tended to strongly enhance the formation and ES-MS detection of sodiated adduct species for a variety of analytes. Since $[TA+Na]^+$ was clearly the most useful species for analytical purposes and would give highest sensitivity, this medium seemed likely to provide advantages for analysis of TA. While 1-propanol is not strictly a nonprotic solvent, its inherent acidity is expected to be significantly lower than that of methanol.[8,9] Ion signals for $[TA+Na]^+$ produced by 1.0 μg mL^{-1} TA in 100% 1-propanol were in fact more intense than those obtained in 100% acetonitrile or 100% methanol (Table 2), while the competing ion signal for $[TA+H]^+$ at *m*/*z* 220 was lowest in 1-propanol.

Formation of $[TA_2+Na]^+$ was also enhanced in 1-propanol. Initially this seemed to discourage use of this solvent, since TA would have to be monitored as two separate adducts, compromising analytical sensitivity. However, the instrument provides the option of source fragmentation, which accelerates ions more rapidly through the moderate-vacuum ion tuning region of the instrument prior to detection. Applying a low level of source fragmentation energy (12–18% of full scale) broke up much of the sodiated dimer, enhancing the ion signal of $[TA+Na]^+$ (Fig. 3). Source fragmentation at 12% full scale was therefore used in all further experiments unless noted.

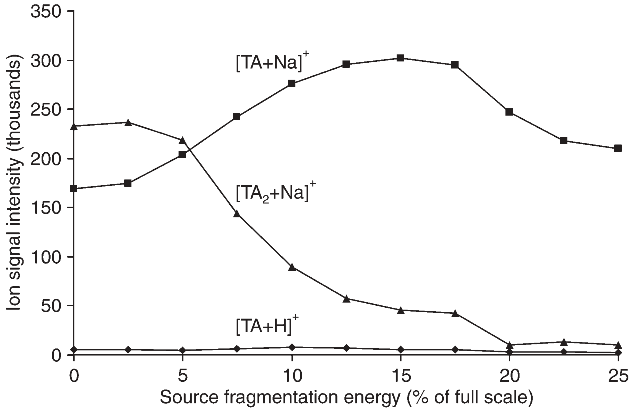

Fig. 3 Effect of source fragmentation energy on ion signals for TA species. Sample is 1.0 μg mL^{-1} TA in 99.9% 1-propanol with 0.10% NH_4OH (v/v), infused at 25 μL min^{-1}. $[TA+H]^+$ is visible at *m*/*z* 220, $[TA+Na]^+$ at *m*/*z* 242, and $[TA_2+Na]^+$ at *m*/*z* 461.

In our instrument, a heated capillary promotes evaporation of any remaining solvent in the electrosprayed sample during passage from the atmospheric-pressure region into the moderate-vacuum ion tuning region. As indicated in Fig. 4, capillary temperature proved to have considerable impact on the observed ion signals. Lower temperatures minimized sodiated adduct signals in favor of the protonated TA ion, while higher temperatures maximized adducts. This trend was observed regardless of choice of solvent or presence of base (see below), though actual ion ratios were affected by these parameters. The results suggest that protonated and sodiated TA species actually continued to coexist in equilibrium in solution. (As described below, this equilibrium apparently may be altered using additives like sodium ion.) It is assumed that during analysis, higher desolvation capillary temperature more efficiently destroyed the less stable protonated form of TA, while survival of the more stable sodiated adducts was enhanced as solvent evaporation became more efficient. At temperatures above 150 °C, $[TA_2+Na]^+$ also declines; we speculate that the dimer is less stable than the monomer and increased thermal energy may be driving one of the TA molecules away before solvent evaporation is complete, leaving $[TA+Na]^+$. The free TA may then be destroyed by the heat or may complex with unbound sodium ion to yield more $[TA+Na]^+$. That $[TA_2+Na]^+$ responds similarly to both increasing source fragmentation energy and temperature further suggests that dimer stability is at issue. Even at low capillary temperature, the intensities of the $[TA+H]^+$ signals were less than those obtained for $[TA+Na]^+$ at higher temperatures, so optimizing for the latter provides the best analytical sensitivity. Capillary temperature was held at 200 °C in all remaining experiments.

Table 2 Relative ion intensities for $[TA+Na]^+$ and $[TA+H]^+$ as a function of solvent media and base concentration[a]

	Relative ion intensity (%)[b]			
	$[TA+Na]^+$ (*m*/*z* 242)		$[TA+H]^+$ (*m*/*z* 220)	
Solvent media	No added base	With 0.5% NH_4OH (v/v)	No added base	With 0.5% NH_4OH (v/v)
Methanol	5.03	20.9	0.353	0.522
Acetonitrile	14.3	24.4	0.856	3.17
1-Propanol	97.8	100.0	ND[c]	1.00

[a] TA (1.0 μg mL^{-1}) was infused at 25 μL min^{-1} and integrated for 60 s in MS scan mode (*m*/*z* 215–260). [b] Ion intensities are relative to 3.60×10^5 ion counts observed for [TA + Na]$^+$ with 0.5% NH_4OH (v/v) in 1-propanol. [c] ND = not detected.

It was observed empirically that standards prepared in 1-propanol proved more stable over time than those prepared in acetonitrile, especially at lower concentrations. Further experiments were nonetheless conducted in both solvents because even the best available grade of 1-propanol exhibited considerably more intense background ion signals in ES-MS than did other solvents. In addition, its higher viscosity may complicate eventual application of HPLC for analysis of TA in

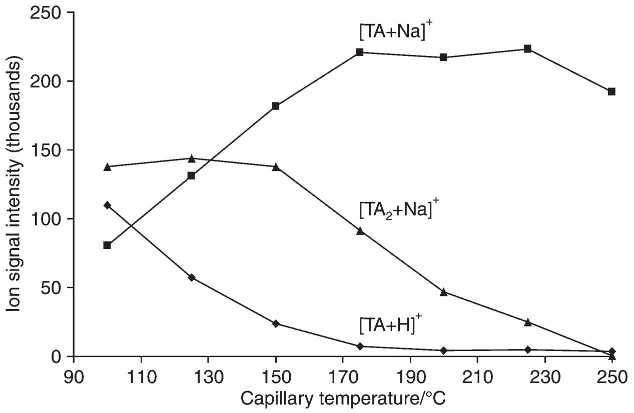

Fig. 4 Effect of desolvation capillary temperature on TA ion signals. Sample is 1.0 μg mL^{-1} TA in 99.9% 1-propanol with 0.10% NH_4OH (v/v), infused at 25 μL min^{-1}. Source fragmentation energy was 12%. $[TA+H]^+$ is visible at *m*/*z* 220, $[TA+Na]^+$ at *m*/*z* 242, and $[TA_2+Na]^+$ at *m*/*z* 461.

complex samples. The use of isopropanol was also investigated as it is less viscous and available in higher purity, but TA was not detectable in any form using this solvent.

Influence of solvent additives

Table 2 compares the effect of base (as percent concentrated ammonium hydroxide) on ES-MS responses for $[TA+H]^+$ and $[TA+Na]^+$ for three solvents. It is apparent that the presence of base enhances $[TA+Na]^+$ signals in some solvent media, presumably by consuming free protons present in the solvents and decreasing analyte loss *via* formation of $[TA+H]^+$. Experiments were also conducted with standards of 1.0 µg mL^{-1} TA prepared in acetonitrile and 1-propanol with ammonium hydroxide varied from 0 to 1.0% (v/v) in 0.10% increments. Standards were introduced by infusion at 25 µL min^{-1} and source fragmentation was not applied so that the dimer could also be monitored. In 1-propanol media, ion signals for $[TA+Na]^+$ and $[TA_2+Na]^+$ were essentially constant at all percentages of base, with the dimer approximately half the intensity of the monomer. In acetonitrile media, $[TA+Na]^+$ signal rose significantly for up to 0.30% base, then was roughly constant for remaining base levels at roughly half the intensity observed in 1-propanol, while $[TA_2+Na]^+$ mirrored the same behavior at much lower intensity (~1% of the $[TA+Na]^+$ signal). Biocide response in acetonitrile thus varied considerably with level of base, but there was relatively little impact in 1-propanol. This probably indicates enhanced formation of sodium adducts in 1-propanol regardless of alkalinity. The fact that 1-propanol also generally gave a higher proportion of $[TA_2+Na]^+$ suggests that it is less stable than the monomer and requires a supportive solvent to form at significant levels.

We subsequently explored the use of known concentrations of sodium ion to promote the formation of $[TA+Na]^+$ in order to enhance analyte stability and analytical sensitivity. The effect of sodium ion concentration on the ES-MS response for the analyte was investigated using standard solutions of 500 ng mL^{-1} TA prepared in either 99.25% acetonitrile or 1-propanol, 0.75% ammonium hydroxide (v/v), and varying amounts of sodium acetate (added as a methanolic solution so as to exclude water). The experiment was conducted with and without source fragmentation, though results for only the former are shown in Fig. 5. In acetonitrile, signals for both sodium adduct ions peaked at 25 µmol L^{-1} Na$^+$ and then fell, while in 1-propanol the signals increased steadily with Na$^+$ level up to 50 µmol L^{-1}, with little further gain at higher levels. The experiment was also conducted with 99.9% 1-propanol/0.10% ammonium hydroxide; adduct ion signals peaked at 75 µmol L^{-1} Na$^+$, but maximum signal intensity was the same as for 50 µmol L^{-1} Na$^+$/0.75% ammonium hydroxide. The $[TA+Na]^+$ ion signals were significantly stronger in 1-propanol than in acetonitrile, while the ratio of $[TA+Na]^+$ to $[TA_2+Na]^+$ was higher in 1-propanol (this was true even prior to application of source fragmentation to maximize the monomer). High levels of sodium acetate were also observed to build up on the entrance of the instrument desolvation capillary when using acetonitrile, while using 1-propanol seemed to minimize or eliminate this problem.

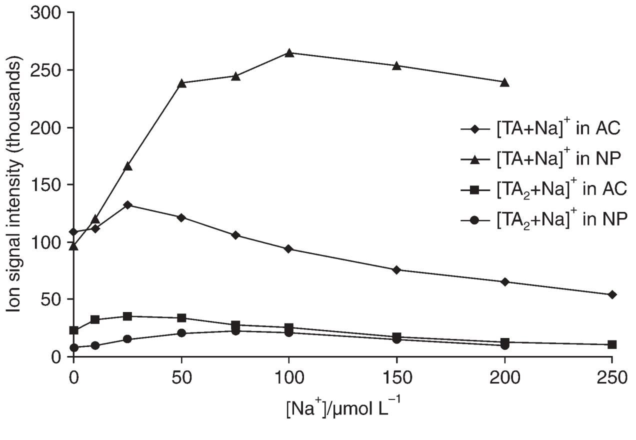

Fig. 5 Effect of Na$^+$ concentration on TA sodium adducts in 99.25% acetonitrile (AC) or 99.25% 1-propanol (NP), both with 0.75% NH_4OH (v/v), infused at 25 µL min^{-1}. Source fragmentation energy was 12%. Na$^+$ was added as sodium acetate in methanol.

The potential use of other alkali metals was also investigated by preparing standards of 1.0 µg mL^{-1} TA in 99.9% 1-propanol/0.1% base with 0, 10, 50, and 100 µmol L^{-1} of Li$^+$, Na$^+$, and K$^+$, again using methanolic stock solutions of the metal acetates and analyzing with and without source fragmentation so that dimer adduct formation could be observed. All three of the alkali metals formed both $[TA+M]^+$ and $[TA_2+M]^+$, which under source fragmentation behaved as the sodium adducts had. For all added metal ion levels, the intensity of $[TA+Na]^+$ was higher than that of $[TA+Li]^+$ and much higher than that of $[TA+K]^+$. The residual sodium ion in the solvent gave considerable $[TA+Na]^+$ signal even when no sodium was added, and this adduct remained the most intense ion species even for TA standards containing 10 µmol L^{-1} Li$^+$ or K$^+$.

The effect of partly aqueous media on TA stability was investigated, since HPLC may eventually be needed to separate the analyte from other components in MWF and chromatographic retention of $[TA+Na]^+$ in neat organic media is expected to be minimal. Solutions of 1.0 µg mL^{-1} TA in organic–aqueous mixtures (all with 0.10% ammonium hydroxide) were prepared and analyzed *via* infusion. The extent of analyte degradation was calculated in comparison to the adduct ion signal in 99.9% organic solvent/0.10% ammonium hydroxide. In acetonitrile/water mixtures, even 5% water rapidly destroyed more than 95% of the TA, while 20% water eliminated more than 99% of it. In 1-propanol/water mixtures, survival of 1.0 µg mL^{-1} TA was somewhat improved when samples were analyzed immediately, although even 5% water still caused approximately 20% TA loss and 10% water caused 64% loss. Further loss was apparent in all organic–water mixtures when the samples were rerun four and nine days later. These results suggest that obtaining HPLC separation for TA using organic–water media may not be feasible, although nonprotic organic mixtures may address the issues (see the concluding discussion below).

Linearity of TA adduct response

The linearity of $[TA+Na]^+$ response *versus* analyte concentration was investigated in flow injection experiments using the HPLC pump to drive solvent, omitting any chromatographic column and running the connective tubing directly from the autosampler injection valve to the detector. This allowed us to observe the behavior of TA adducts at a flow rate (230 µL min^{-1}) which would be compatible with HPLC-ES-MS. HPLC was not explored at this stage because of concerns about high back pressure generated by the viscosity of 1-propanol, the likelihood of inadequate analyte retention using 100% organic media, and uncertainty as to whether even the stabilized biocide could survive passage through the high-surface-area environment of an LC column.

For initial trials, both acetonitrile and 1-propanol media were used, although the latter was explored more extensively. All samples in acetonitrile contained 0.75% base and up to 25 µmol L^{-1} Na$^+$, while samples in 1-propanol were prepared with and without 0.10% base and up to 50 µmol L^{-1} Na$^+$. Levels of OH$^-$ and Na$^+$ in samples and in the blank solvent delivered by the HPLC pump were always matched to avoid biasing analyte peak response. TA standards were prepared by serial dilution at levels ranging from 5 ng mL^{-1} to 2 μg mL^{-1}. The flow injection peak heights for three or four replicate injections were averaged for each level. Polypropylene autosampler vials were used to avoid additional contribution of Na$^+$ to standards while they were in the analysis queue. Data for these trials were collected by monitoring only [TA+Na]$^+$ at *m*/*z* 242 for best sensitivity and precision.

Linear response was difficult to obtain in acetonitrile unless Na$^+$ was added, although performance still remained erratic. This may indicate that in absence of a supportive solvent, the TA adduct does not reliably survive transport through the connective tubing. Linearity was generally better in 1-propanol, where [TA+Na]$^+$ apparently formed efficiently over the observed TA concentration range even without addition of Na$^+$ due to residual sodium in the solvent. Although plots were reasonably linear up to 1.50 μg mL^{-1} TA in the absence of sodium, it became apparent that increasing levels of Na$^+$ sharply limited the linear dynamic range. This might have been a concomitant effect; as the amount of ionic additive increases, it may reduce the efficiency of [TA+Na]$^+$ ion generation and of spray desolvation, so TA peak intensities plateau at lower concentrations. However, instrumental sensitivity may also have drifted over the period of time needed to collect the data, an effect exacerbated by high levels of Na$^+$.

All prior studies had been performed without use of an internal standard, which would be expected to improve quantitative performance of the method, especially regarding linear response *via* flow injection if instrumental drift was an issue. The ideal internal standard in this case would probably be a sodiated species with equilibrium behavior approximating that of TA itself, but we could not locate such a compound. As a candidate internal standard, we selected *N*,*N*-dimethyloctylamine (DMOA), a strong base that protonated adequately even in alkaline 1-propanol and was thus visible at *m*/*z* 158. Twenty-two standards covering the range from 0 to 1.50 μg mL^{-1} TA were prepared and each contained 100 ng mL^{-1} DMOA. The high number of standards allowed time for instrumental drift to become manifest. These standards were analyzed as described above (1-propanol media only), except that scan mode was used (*m*/*z* 150–250) and the internal standard ion intensity was also recorded. The experiment was performed first with no added base or sodium, then with 0.10 and 0.50% (v/v) of ammonium hydroxide plus 0, 10, or 50 μmol L^{-1} Na$^+$. For each solvent medium, calibration plots were made using both the average height of TA peaks and the response ratio (average peak height of TA adduct/average peak height of DMOA ion).

The correlation coefficients of the resulting plots indicated that in this concentration range, TA response was generally more linear when fewer additives were present in the solvent media, although the presence of a low level of base was desirable: correlation coefficients were relatively poor when neither base nor sodium ion were added (and actually worse for the response ratio plot) while the best were obtained with 0.10% base only (0.9978 for TA peak heights, 0.9991 for response ratio). Using the response ratio instead of TA peak height demonstrably improved linearity in most cases, with greatest improvement generally observed for solvent media containing the highest levels of additives. The worst plots exhibited marked curvature or plateau at higher TA concentrations (usually over 1.0 μg mL^{-1}) and no correlation coefficients were calculated for these over the full concentration range. However, examination of these plots and subsequent linear regression on limited portions of the data showed that many of them were approximately linear in low concentration ranges, as had been observed for the earlier calibration data obtained without internal standard present. The first nine calibration standards (0 to 50 ng mL^{-1}) were rerun, this time monitoring only [TA+Na]$^+$ at *m*/*z* 242 in order to improve sensitivity and precision at the lowest levels. Results using average TA peak height gave correlation coefficients ranging from 0.9939 to 0.9992 in all cases, comparable or greatly improved linearity relative to that for corresponding full range plots, and with less overall variation. Limits of detection (LODs) calculated using these regression plots (LOD = three times the standard error of regression divided by the slope) ranged from 1.6 to 4.3 ng mL^{-1} TA. Neither low-range linearity nor detection limits were clearly correlated with the presence or levels of ammonium hydroxide or Na$^+$.

Detection of TA in metalworking fluids

Two unused MWF obtained from a machining plant (hereafter called samples I and II) were used to demonstrate the efficacy of this method for direct detection of TA. The compositions of these fluids are proprietary, but their mineral oil content places them in the general MWF class termed semisynthetic. To minimize viscosity and concomitant interferences from other MWF components, aliquots of each fluid were diluted 10000-fold in 99.9% 1-propanol/0.10% ammonium hydroxide. When the diluted samples were observed in ES-MS *via* infusion, no ion signal was visible at *m*/*z* 242, indicating that the MWF at this point contained no detectable levels of TA (Fig. 6A). Each MWF was then spiked with 5000 μg mL^{-1} TA, a level that might typically be added to MWF in the field. Aliquots were again diluted 10000-fold in the alkaline 1-propanol, giving a final concentration of 500 ng mL^{-1} TA. Ion signals for the [TA+Na]$^+$ adduct were easily observed (Fig. 6B) and identity was confirmed by comparing the collision-induced fragment ion pattern with that of a 500 ng mL^{-1} TA standard prepared in the alkaline 1-propanol. Ion signal intensity for [TA+Na]$^+$

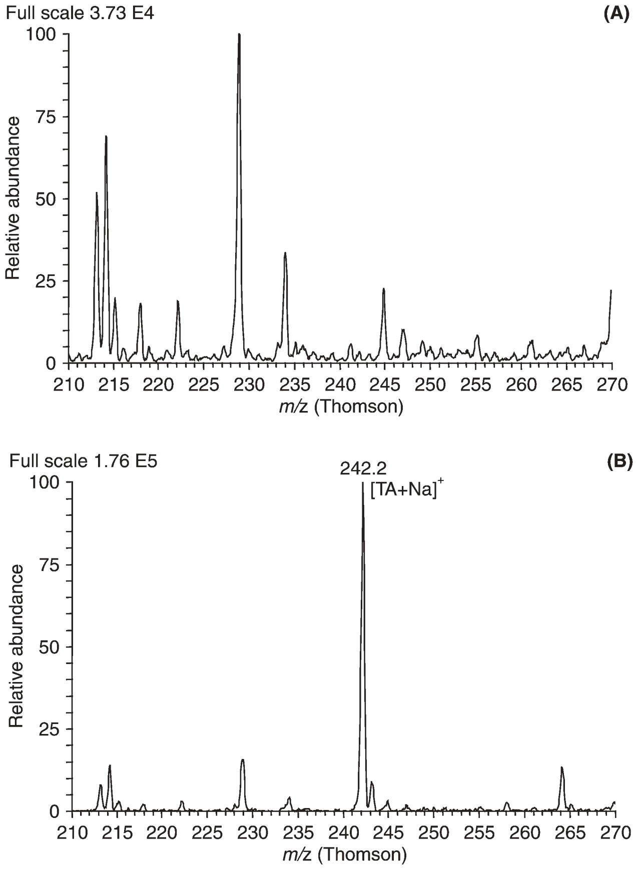

Fig. 6 (A) Metalworking fluid sample I diluted by 10000 in 99.9% 1-propanol/0.10% NH_4OH (v/v). (B) Same fluid spiked with 5000 μg mL^{-1} TA and diluted by 10000 in same media. Signal for [TA+Na]$^+$ is visible at *m*/*z* 242. Other ion signals are due to unidentified sample components or solvent impurities.

in the diluted MWF was only 57% of that for the standard, probably due to remaining matrix concomitant effects. Thus, while TA in MWF may be directly observed in ES-MS using a simple dilution step, the method of standard addition will likely be needed for reliable quantification. An internal standard to compensate for instrumental drift is also desirable. These dilutions of TA-spiked MWF were refrigerated and periodically rerun, and even after four months the $[TA+Na]^+$ ion remained visible with little decrease in intensity. This suggests that diluting MWF with alkaline 1-propanol effectively "fixes" low levels of TA, which may promote preservation during sampling in the field.

Later, the experiment was repeated on another unused semisynthetic MWF (sample III), for which the MSDS indicated a significant proportion of TA (1–10%) as manufactured. The fluid was diluted 10000-fold in alkaline 1-propanol as above, and was then prepared for standard addition analysis using four increments of 100 ng mL^{-1} TA, for a total of five data points (the TA standard that was added was itself prepared in alkaline 1-propanol to avoid altering sample composition). DMOA at 300 ng mL^{-1} was incorporated as internal standard. Samples were infused at 25 μL min^{-1}, with blank solvent run after each to clear transfer lines and minimize sample carryover, and the intensity of the ion signal at *m*/*z* 242 after 1 min integration was plotted. The first, unspiked sample exhibited a signal for TA at *m*/*z* 242, and collision-induced fragmentation of this precursor ion yielded the correct pattern of fragment ions as described above. The standard addition regression plots were linear for adduct peak height and response ratio (correlation coefficients = 0.99840 and 0.99890 respectively) and indicated TA concentrations of 145 and 139 ng mL^{-1}, equivalent to 1450 or 1390 μg mL^{-1} prior to dilution. This is well below the level quoted on the MSDS. However, the broad range listed for TA suggests that only an estimate of actual content was available, while the age of the sample and degree of subsequent TA loss prior to analysis are unknown. The experiment nonetheless further demonstrates direct identification and quantification of TA in a field MWF sample.

New 10000-fold dilutions of MWF samples I and II that had been spiked with 5000 μg mL^{-1} TA were also prepared and analyzed over a three-week period. Signals for $[TA+Na]^+$ continued to be visible both immediately after preparation and after prolonged refrigerated storage. This was somewhat surprising, since these fluid samples contained >90% water, which would be expected to degrade TA. To test this, samples of 1000 μg mL^{-1} TA were prepared in water buffered to pH values ranging from 4 to 11 (in increments of 1.0 pH unit) using 0.010 mol L^{-1} ammonium acetate with acetic acid or ammonium hydroxide. These were further diluted 1000 times in alkaline 1-propanol to give 1.0 μg mL^{-1} TA (assuming no analyte degradation). $[TA+Na]^+$ ion signals were compared against those for a 1.0 μg mL^{-1} TA standard prepared in neat alkaline 1-propanol. Over 95% of the TA prepared in water buffered to pH 11 survived, but only 32% remained at pH 10, and loss was essentially complete at pH 9 and below. Many MWF are prepared with considerable alkaline reserve to counteract metal corrosion,[1] and the pH of MWF samples I and II ranged between pH 9 and 11 when tested with colorimetric strips. The survival of TA in aqueous media may also be influenced by the presence of sodium ion, which is typically present at high levels in many MWF. Experiments that explore this possibility are planned.

Future investigations

Ideally, it will be possible to separate TA from concomitants entirely *via* liquid chromatography, although it is unlikely that neat 1-propanol, in which the analyte adduct appears to survive best, could be used as mobile phase. HPLC using binary phases containing low percentages of 1-propanol has been demonstrated.[10] It may be possible to use a mobile phase combining 1-propanol with some nonprotic solvent (possibly acetonitrile) that minimizes viscosity but allows the TA adduct to survive long enough for HPLC-ES-MS detection. In this context, the possibility that Na^+ can promote survival of TA in samples despite the presence of protic solvent merits investigation; adding known amounts of Na^+ to a binary mobile phase may counteract any tendency for TA to degrade during analysis, while using an internal standard may largely compensate for the impact of the additive on linearity of response.

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