Environmental applications of LC/MS/MS and LC/MS/Linear Ion Trap MS

Robert I Ellis[1], Takeo Sakuma[1], Andre Schrieber[1], Houssain El Aribi[1]

[1]MDS Sciex/Applied Biosystems

Introduction

Liquid Chromatography interfaced to Tandem Mass Spectrometry (LC-MS-MS) is a growing technique for environmental analysis, including persistent organic pollutants, halogenated species, polycyclic aromatic hydrocarbons and pesticides. The technique is also being implemented in monitoring and assessment of emerging contaminants such as fluorinated surfactants, perchlorate and brominated flame retardants. LC-MS-MS offers advantages in sensitivity, selectivity, sample preparation and robustness over conventional analytical techniques, such as liquid chromatography with UV detection and Gas Chromatography. LC-MS-MS is applicable to polar and non-polar analytes, often without derivatization or pre-concentration.

The aim of this study was to demonstrate LC-MS-MS for analysis of several diverse chemical species that are known to challenge conventional methods. Applications include both polar and non-polar species. Both quantitative and qualitative LC-MS-MS techniques were demonstrated together with special techniques such as photoionization, ion chromatography, and software techniques such as library creation and searching.

The applications that were investigated are : haloacetic acids in drinking water, perchlorate in waste water and wine, pesticides in soil and water, and polycyclic aromatic hydrocarbons.

Haloacetic acids are potentially formed during the chlorination of drinking water. Haloacetic acids (HAAs) can be considered as carcinogenic promoters in that they have hepatotoxic and neurotoxic effects on animals. Bromoacetic acids have an even more adverse effect than chloroacetic acids because they combine oxidative action to a halogenating action. Both chlorine and bromine-containing acetic acids are potentially formed during the chlorination disinfection of drinking water. These analytes are amenable to MS-MS analysis for qualitative and quantitative purposes but because of their high polarity traditional LC separation can be difficult. In this example we used ion chromatography, a separation technique that is well suited to separation of highly polar ions, interfaced to MS-MS for quantitation.

Perchlorate became a well-publicized environmental contaminant in the spring of 1997 when it was been found in over 500 drinking water supplies in at least 20 states, serving well over 20 million people in USA. In 2002, the EPA recommended a maximum containment level (MCL) for perchlorate of 1 µg/L (ppb) in drinking water. Some states have set their own limits ranging from 4 to 18 ppb and California’s current “notification level” is 6 ppb. Perchlorate contamination is a concern for several reasons. First, there are uncertainties in the toxicological database that is used to address the potential of perchlorate to produce human health effects when present at low levels in beverages and food products. Additionally, the actual extent of perchlorate occurrence in ground and surface waters and other media (soils or plant and animal tissues) is unknown — a problem compounded by limits to the analytical detection method. Separation of the highly polar perchlorate anion is amenable to Ion Chromatography coupled to MS-MS for selective and sensitive detection. In this study perchlorate was analyzed in foods and in water samples.

More than 800 pesticides are used worldwide. For many of these compounds legal action levels (e.g. maximum residue limits or tolerances) in food exist, which have to be controlled. For this type of target analysis, multi-residue analytical methods are preferred to reduce the workload. There are several problems with routine pesticide analysis – some compounds are not amenable to the traditional GC-MS analysis, and many samples contain few pesticides so a screening method is applicable. In this study LC-MS-MS with conventional reverse phase chromatography was used to determine up to 300 pesticides and metabolites. In addition to quantitation, triggered MS-MS scans were performed to identify pesticides. The MS-MS scans were compared to a comprehensive spectral library for confirmation.

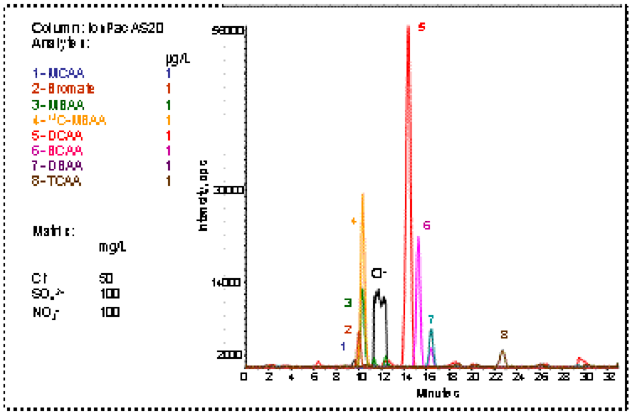
Polycyclic aromatic hydrocarbons (PAH) are a group of over 100 different non-polar chemicals, typically formed during incomplete combustion, which are present in a variety of environmental matrices. Airborne PAH's, usually found in aerosol particles, are implicated in heterogeneous atmospheric processing. Important carcinogenic PAH's may be carried by ultrafine particles in ambient air into the lungs, and are therefore targets of rigorous environmental monitoring. Currently, environmental PAH's are most commonly analyzed by HPLC or GC/EI-MS; techniques which require long-high-resolution separations, and complicated MS methods. Photoionization is a technique that uses the energy of photons to generate ions from the vaporized eluent of an LC, and is well suited to non-polar species. This allows simple routine measurement of PAH's by LC-MS-MS.

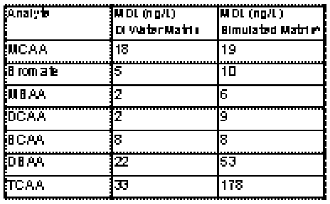
Materials and Methods

This work was carried out on Applied Biosystems/MDS Sciex mass spectrometer models API 2000™, API 3200™, 3200 Q TRAP®, API4000™ and API 5000™. The instruments were used with TurboIonSpray® and PhotoSpray™ sources. Liquid chromatography was carried out using Agilent 100 liquid chromatography pumps and autosamplers, and water/acetonitrile mobile phases with ammonium acetate and formic acid modifiers. Various 2.1mm and 4.6mm internal diameter reverse phase columns were used. Ion Chromatography was carried out using a Dionex 2500 Ion Chromatography system with potassium hydroxide eluents. The flow rates were in the range 200 to 1000 µl/min.

Results and Discussion

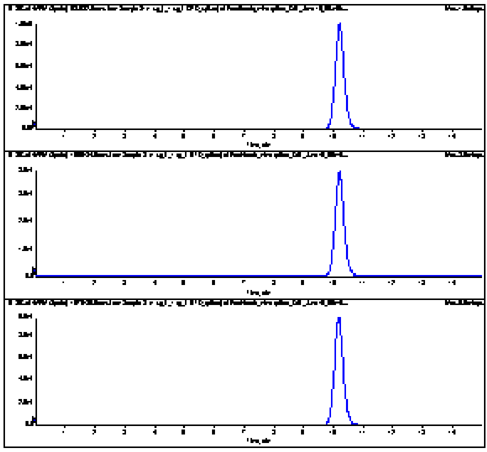
Haloacetic acids including monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), dibromoacetic acid (DBAA), trichloroacetic acid (TCAA), tribromoacetic acid (TBAA), chlorodibromoacetic acid (CDBAA), dichlorobromoacetic acid (DCBAA) and chlorobromoacetic acid (CBAA) were diluted and spiked with a simulated high-salt matrix as indicated in the figure below. These samples were analyzed by Ion Chromatography MS-MS. All analytes were separated in the ion chromatography method and each compound was analyzed by using a specific multiple reaction monitoring (MRM) MS-MS transition on an API 3200™. This is the most highly specific and sensitive method of MS-MS analysis. Linear calibration curves were prepared and the limit of detection calculated as in the table below.

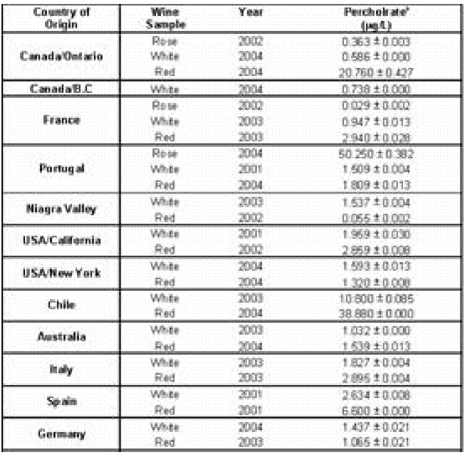




Analyte	MDL (ng/L) DI Water Matrix	MDL (ng/L) Simulated Matrix
MCAA	18	19
Bromate	5	10
MBAA	2	6
DCAA	2	9
BCAA	8	8
DBAA	22	53
TCAA	33	178

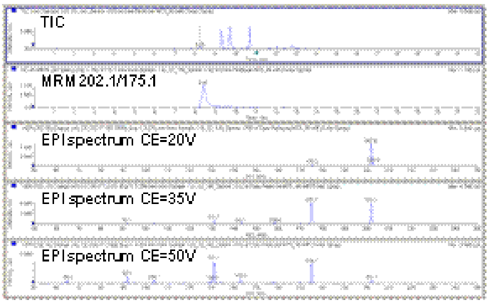
Multiple Reaction Monitoring (MRM) mode was also used to quantitate perchlorate anion in wine samples using an API 3200™ or an API 2000™ instrument. The MRM transitions that were monitored are: $^{35}Cl^{16}O_4^-$ (m/z 98.8) / $^{35}Cl^{16}O_3^-$ (m/z 82.8), $^{37}Cl^{16}O_4^-$ (m/z 100.9) / $^{37}Cl^{16}O_3^-$ (m/z 84.8) and $^{35}Cl^{18}O_4^-$ (m/z 107.0) / $^{35}Cl^{18}O_3^-$ (m/z 89.0). In this method the first transition was used for quantitation and the second is used for confirmation. The measured isotopic ratio of ^{37}Cl to ^{35}Cl and the comparison between standard and sample areas ratios were used to confirm the presence of perchlorate and to determine any interference that may cause systematic error in detection. The figures below show the chromatograms for 1 ppb perchlorate in a wine matrix perchlorate using MRM transitions ClO_4^-/ClO_3^- for the Cl^{35}, Cl^{37} and O^{18} internal standards. The limit of detection was 1ppb on the 3200 Q TRAP®. The table below shows levels of perchlorate in wine samples.

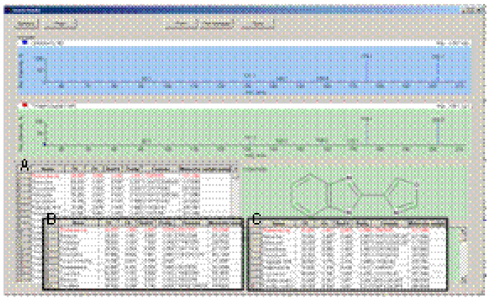




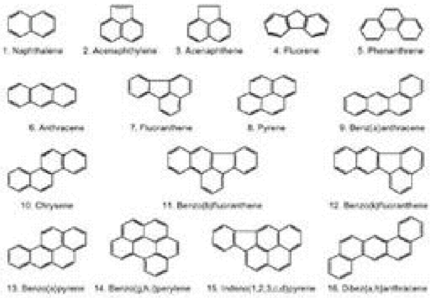
Country of Origin	Wine Sample	Year	Perchlorate' (µg/L)
Canada/Ontario	Rose	2002	0.363 ± 0.003
	White	2004	0.586 ± 0.000
	Red	2004	20.760 ± 0.427
Canada/B.C	White	2004	0.738 ± 0.000
France	Rose	2002	0.029 ± 0.002
	White	2003	0.947 ± 0.013
	Red	2003	2.940 ± 0.028
Portugal	Rose	2004	50.250 ± 0.382
	White	2001	1.509 ± 0.004
	Red	2004	1.809 ± 0.013
Niagra Valley	White	2003	1.537 ± 0.004
	Red	2002	0.055 ± 0.002
USA/California	White	2001	1.969 ± 0.030
	Red	2002	2.859 ± 0.008
USA/New York	White	2004	1.593 ± 0.013
	Red	2004	1.320 ± 0.008
Chile	White	2003	10.800 ± 0.085
	Red	2004	38.880 ± 0.000
Australia	White	2003	1.032 ± 0.000
	Red	2004	1.539 ± 0.013
Italy	White	2003	1.827 ± 0.004
	Red	2003	2.895 ± 0.004
Spain	White	2001	2.634 ± 0.008
	Red	2001	6.600 ± 0.000
Germany	White	2004	1.437 ± 0.021
	Red	2003	1.065 ± 0.021

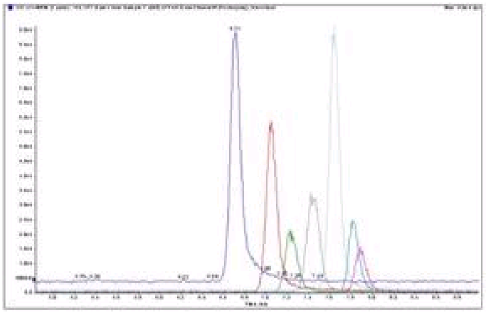
A reverse-phase LC-MS-MS method was used to separate 300 pesticides using MRM methods of detection. Screening for pesticides can be accomplished by examining the MRM chromatograms for each analyte. A feature of the 3200 Q TRAP® is the in-built linear ion trap which allows the user to collect both qualitative and qualitative data in the same LC run, therefore both quantifying and confirming the presence of the analyte. The figures below show the total ion chromatogram (TIC), extracted ion chromatogram for Thiabendazol in a lemon sample, and also the library searching results for this compound. Also shown are the extracted ion chromatogram for the MRM transition, and several MS-MS spectra at different collision energies. Data was also collected for undiluted water sample analysis on an API 5000™





PAH compounds are non-polar and therefore difficult to ionize in LC-MS-MS. For this application a PhotoSpray™ source was utilized with a normal phase separation method. In the chromatogram below six PAH compounds are shown at a level of 100 ppb. The compounds are measured by specific MRM transitions. With this method a limit of detection around 1ppb was achieved using an API 4000™ instrument.





Conclusion

In this paper several methods for LC-MS-MS were presented that are suitable for analysis of a variety of compounds which are both challenging and or current environmental interest.