MS Applications Guide

Chromatography Applications with Mass Spectrometric Detection

Ion and Liquid Chromatography • Electrospray Ionization
Atmospheric Pressure Chemical Ionization • Mass Spectrometry

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1. Introduction

Using This Guide

The methods shown in this guide are offered as proof of concept statements and starting points for method development. Limits of detection (LOD) and issues regarding method reproducibility are not presented in this guide.

Mass Spectrometric Detection Using Atmospheric Pressure Ionization for Liquid Chromatography Techniques

Interfacing chromatographic techniques such as high performance liquid chromatography (HPLC or LC) or ion chromatography (IC) and mass spectrometry (MS) provides the separation scientist with a powerful analytical tool. Recent developments in IC/LC with MS have made these techniques some of the most important methods available to detect and characterize compounds. Directly coupling IC/LC with MS offers the following advantages:

- Structural information
- Speed of analysis
- Convenience
- Analysis of multicomponent mixtures
- Accurate quantitation
- Evaluation of chromatographic peak purity

While the potential benefits of interfacing to MS have been clearly recognized for many years, creating a truly automated connect-and-use interface has proven a difficult challenge.

Atmospheric pressure ionization (API) techniques now provide highly sensitive detection using conventional and capillary flow rates on benchtop MS detector systems. These interfaces work with typical solvent and eluent compositions, whether the separation is achieved by isocratic or gradient elution. API using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) interfaces have proven invaluable in meeting sensitivity requirements for quantitative methods. The molecular weight and structural information provided by MS detection are complementary to techniques such as nuclear magnetic resonance (NMR) and infrared spectroscopy (IR).

Atmospheric Pressure Ionization

API is a technique for interfacing the eluent flow from an LC or IC column to a mass spectrometer. The API interface must: 1) separate the analytes from the solvent, 2) ionize the analyte molecules, and 3) maintain vacuum in the mass detector.

Two major incompatibilities exist when combining the methods. First, the eluent stream being liquid (and often aqueous) can flow at >1 mL/min, while the MS operates at a pressure of about 10^{-6} torr (1.3×10^{-4} Pa). Therefore, it is not possible to pump the eluent directly into the source of the mass spectrometer while maintaining the necessary vacuum. Second, the majority of analytes likely to be separated are relatively involatile and/or thermally labile and are not amenable to ionization techniques used in gas chromatography mass spectrometry (GC-MS).

Alternative ionization and interface techniques have been developed to overcome the incompatibilities of LC/IC and MS. Two types of API interfaces are commonly encountered. These are ESI and APCI.

Electrospray Ionization

ESI is an API technique that provides a simple, real time means of analyzing a wide range of polar molecules. The first ESI results were announced in 1984, and the first application to protein analysis came four years later, in 1988.

For smaller molecules (up to 1–2 K Daltons in molecular mass), an $[M+H]^+$ or $[M-H]^-$ ion is detected depending on whether positive or negative ion detection has been selected. This is similar to the traditional chemical ionization (CI) used in GC/MS, but ESI is a much softer ionization technique, producing significantly less fragmentation. In addition, the analyte does not have to be exposed to high temperatures (this is good for thermally labile compounds) or be volatile. Although the mass spectrometer does require the analyte to be in the gas phase and in a charged state (two basic requirements of all mass spectral analytes), this is accomplished without the conventional GC/MS techniques employed for vaporization and ionization.

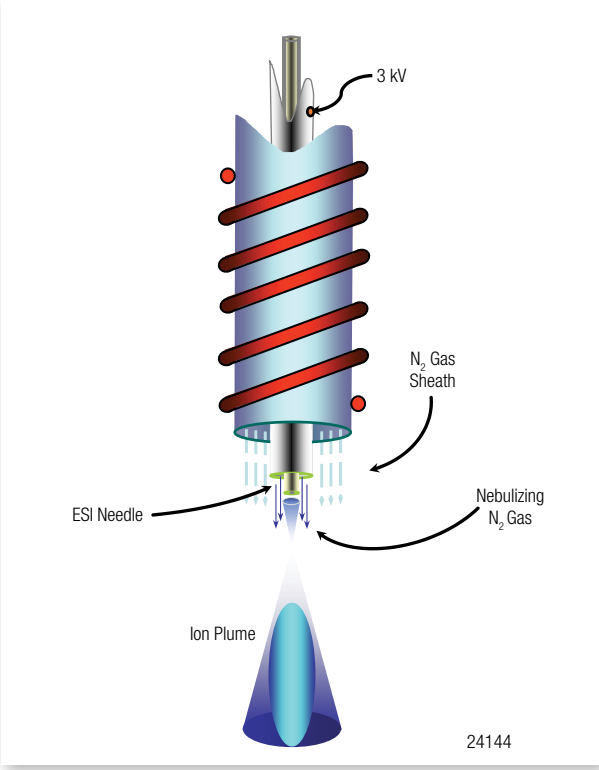


Figure 1-1. Thermally and pneumatically assisted ESI probe

How ESI Works

The mechanism of ESI involves the emission of ions from a droplet into the gas phase at atmospheric pressure, a process known as ion evaporation. The eluent passes through a stainless steel capillary that carries a high potential, typically 2 to 4 kV. The strong electric field generated by this potential and a concentric nebulizing nitrogen gas flow cause the formation of a fine spray of highly charged droplets at the tip of the capillary (hence, ESI).

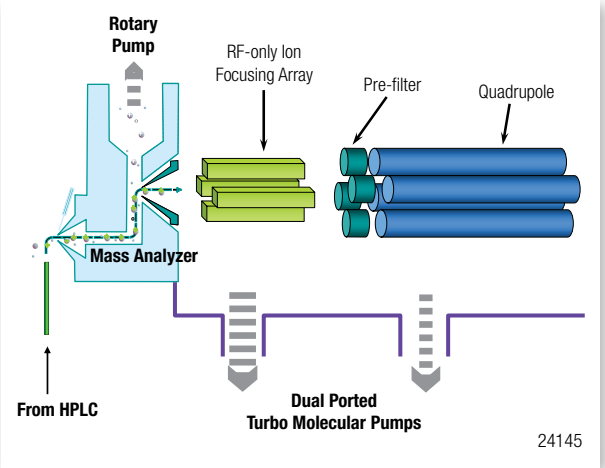


Figure 1-2. Pressure zones

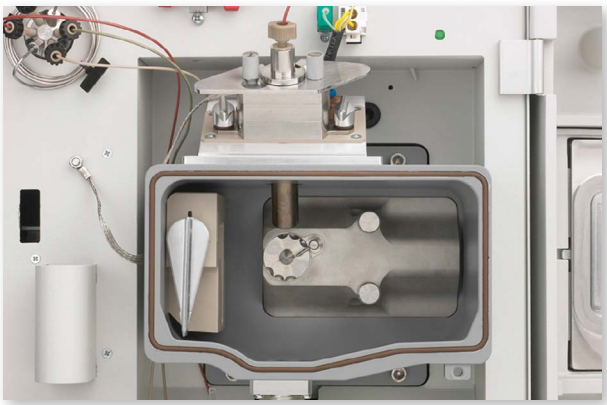


Figure 1-3. API Interface: The API probe carries a high potential with respect to the entrance cone. The source chamber and entrance cone are at the same electric potential which forms a field-free region within the source. The photo above shows an API source with ESI probe in the Thermo Scientific™ MSQ™ mass spectrometry detector. All interface components are maintained in a fixed position, including the probe. The complete system requires very little setup or tuning to achieve optimum sensitivity. Flow focusing eliminates the need for additional electrostatic lenses to focus ions into the source entrance cone and through the exit cone.

Ion Evaporation

The ion evaporation process is assisted by a second concentric flow of heated nitrogen sheath gas. This heating process, which is close to the source entrance cone, enables the routine use of a wide range of liquid flow rates in ESI mode (50 µL to >1 mL min^{-1}). As a sample droplet moves through the heated sheath gas, the solvent evaporates, decreasing the size of the droplet, which increases charge-to-volume ratio. When this ratio reaches the Rayleigh instability limit, the droplet undergoes a coulombic explosion, producing a smaller droplet with a lower charge-to-volume ratio. As more solvent is eliminated, the process is repeated. The end result yields ions in the gas phase. The newly formed ions flow into the entrance cone, pulled by the strong electric field and pressure differential at the entrance orifice. Ions entering the source chamber are then focused into the radio frequency (RF) lens. The RF lens is used to focus the ions before they enter the mass analyzer.

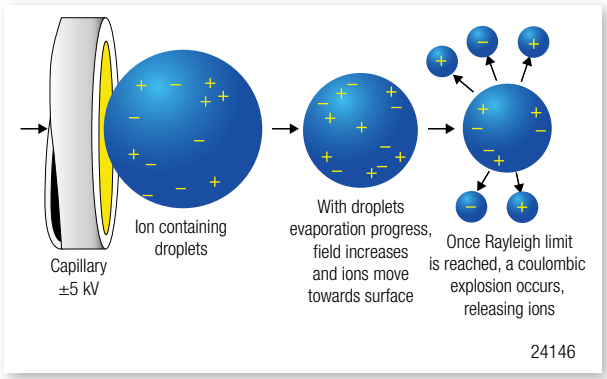


Figure 1-4. Electrospray mechanism

Characteristics of Electrospray Spectra

ESI is a soft ionization technique that typically gives rise to mass spectra dominated by protonated molecules for positive ion analysis and deprotonated molecules for negative ion analysis. This enables molecular weight confirmation from these pseudo molecular ions. Positive and negative ions are detected in general, e.g. K^+, Cl^-, etc.

Polar compounds of low molecular weight (<1000 Daltons) form singly charged ions by the loss or addition of a proton. Basic compounds (e.g., amines) usually form a protonated molecule $[M+H]^+$ that can be analyzed in positive ion mode to give a peak at mass M+1. Acidic compounds (e.g., sulphonic acids) usually form a deprotonated molecule $[M-H]^-$ that can be analyzed in negative ion mode to give a peak at mass M-1.

The ESI technique can be used for molecules with higher molecular masses (up to 200,000 Daltons). Higher molecular weight compounds (such as proteins) can produce multiply charged ions, such as $[M+nH]^{n+}$. Because MS measures the mass-to-charge ratio (*m/z*), if the molecular weight were 10,000 Daltons, a doubly charged ion (2+ in +ve ion) would be seen at 5001 *m/z*, 10+ at 1001 *m/z*, etc.

If ionization takes place in the presence of contamination or additives, such as ammonium or sodium ions, some compounds are susceptible to adduct formation. These spectra show other ions in addition to, or instead of, the molecular ion. Common adducts are formed with ammonium ions, NH_4^+ $[M+18]^+$, sodium ions, Na^+ $[M+23]^+$ and potassium ions K^+ $[M+39]^+$.

Obtaining Structural Information

By increasing the potential on the source block, it is possible to induce diagnostic fragment ions, which are formed between the exit cone and the RF lens, thereby increasing specificity of detection. This is referred to as source fragmentation or collision induced dissociation (CID).

Fragmentation of Ions

The sample ions remain intact in the field-free region – the viscous flow region (>1 mbar) between the entrance and exit cones of the source. However, as the ions pass through the exit cone, the pressure decreases significantly, which in turn increases the mean-free path between collisions with neutral gas molecules. Ions can now gain energy between collisions under the influence of the controlled electric field between the exit cone and the RF lens. If the voltage is kept low, fragmentation is minimal and molecular ions will dominate. Raising the voltage will increase the energy imparted to the ions during collision – this energy may be sufficient to induce ion fragmentation, or *source* fragmentation. As the source voltage is further increased, more fragmentation may occur. Usually, only weaker bonds are broken such as C-N, C-S, and C-O due to the level of energies involved.

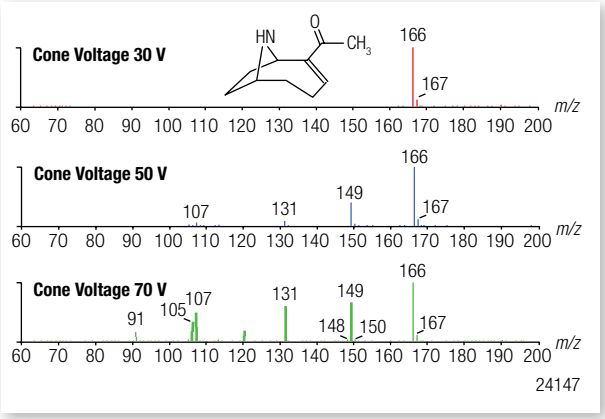


Figure 1-5

Limitations on LC Flow Rate

Traditionally, ESI sources have operated in the LC flow range of 10–200 µL/min. This flow rate limitation required the use of a flow splitter for most applications, which could be cumbersome and unreliable. In contrast, current interfaces operate with flow rates used by analytical (1 mL/min) and microbore columns (250 µL/min).

Types of Compounds can be Analyzed by ESI

Polar compounds or ionic species present in solution (such as in IC). Polar compounds of molecular weight <200,000 are suitable for analysis by ESI.

Typical applications are: low-molecular weight anions and cations, peptides, proteins, oligonucleotides, sugars, and drugs.

Atmospheric Pressure Chemical Ionization

APCI is similar to ESI in many ways; it is a gas phase rather than *liquid phase* ionization technique and it is used when the analytes remain neutral in solution, such as with separations performed using normal phase LC. Ionization takes place at atmospheric pressure in a two-step process, and ions are sampled into the mass spectrometer in the same way as with ESI. The APCI technique uses the ionized mobile phase as the reagent for a gas phase ion-molecule reaction with the neutral analytes via a protonation or charge-transfer reaction. As in ESI, $[M+H]^+$ and $[M-H]^-$ are formed to provide molecular weight information. Fragmentation can be induced as with ESI using source fragmentation.

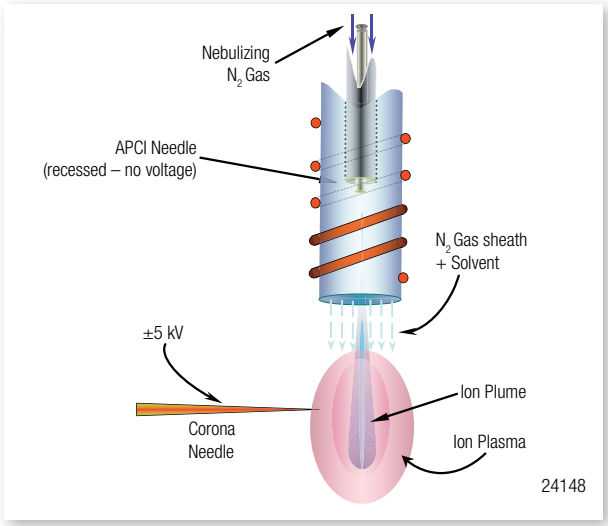


Figure 1-6. Heated APCI probe

How It Works

The LC eluent enters the source via a grounded capillary and is converted into an aerosol by concentric nebulizing and sheath nitrogen gas flows. The mobile phase and sample are converted into the gas/vapor phase by the APCI heater (held at 200–550 °C). A high potential (3–5 kV) is applied to a discharge needle in the source, forming a corona discharge. This Thermo Scientific Dionex™ Corona™ Charged Aerosol Detector consists primarily of the mobile phase. Ionization of the neutral analyte molecules occurs via gas phase ion-molecule reactions with the ionized mobile phase in the corona discharge. Ion samplings into the mass analyzer and fragmentation mechanisms are the same as those described for the ESI interface. The APCI technique is rugged and is far less dependent on solvent choice, flow rates, or additives as compared to ESI. In addition, ESI is a concentration dependent technique, whereas APCI is a mass sensitive ionization technique, which means it operates well regardless of mobile phase flow rates.

Effect of the Heated Probe on Thermal Decomposition

The APCI technique requires that the analyte be in the vapor phase; therefore, the probe temperature is critical. Higher temperature is required to desolvate and vaporize the analyte and the mobile phase compared to ESI. Although a high temperature is applied to the probe, most of the heat is used to evaporate the solvent and heat the nitrogen gas. The thermal effect on the sample is minimal, however, with thermally labile (unstable) compounds, the heated probe may cause some thermal fragmentation.

Characteristics of APCI Spectra

The APCI spectra are similar to those in ESI (i.e., positive ion mode $[M+H]^+$, negative ion mode $[M-H]^-$) although thermal fragment ions are more likely to occur. Unlike ESI, APCI does not produce multiply charged ions and is unsuitable for the analysis of high molecular weight compounds such as proteins. In some cases, APCI can also produce adduct ions in the presence of sodium or ammonium ions.

Obtaining Structural Information

As with ESI, structural information can be obtained by using source fragmentation with the CID process.

Limitations on LC Flow Rate

Flows rates from 200 µL to 2.0 mL/min. can be used with APCI, allowing the direct coupling of 2.1 mm and 4.6 mm i.d. HPLC columns to the APCI interface.

Types of Compounds Analyzed by APCI

APCI is used when the analyte contains no acidic or basic sites (e.g., alcohols, aldehydes, esters, ketones, hydrocarbons, etc.). APCI can also analyze polar compounds – specifically those much less polar than those detected using ESI. Analytes of molecular weight <1000 are suitable for analysis by APCI.

Buffers and Other Eluent Additives

Historically, liquid chromatography mass spectrometry (LC-MS) has been compatible only with volatile buffer systems, using modifiers such as trifluoroacetic acid, formic acid, and acetic acid. Phosphate buffers, although used extensively in LC separations, were not suited to MS detection, due to rapid blocking of the ion sampling region caused by the deposition of involatile phosphate salts.

Due to the destructive nature of MS, when suppressed conductivity ion chromatography is used prior to introduction to the MS the suppressor must be run using external water mode as opposed to autosuppression mode. Chemical regenerants are discouraged because they can introduce a higher background signal making low-level determinations difficult.

When an API probe is in use, most of the eluent is directed away from the inlet orifice. Neutral eluent or insufficiently ionized droplets are removed. In theory, only analyte ions are attracted to the entrance cone to be swept into the MS. However, under typical LC-MS conditions, both the ions and the charged liquid droplets (containing involatile components) are deflected by the electric field towards the inlet orifice.

This effect leads to a gradual buildup of involatile compounds and electrical insulation, with a commensurate loss in sensitivity over time. The MSQ mass spectrometry detector incorporates a self-cleaning API interface. It is compatible with existing methods – especially those including salts, ion-pairing agents, and plasma extracts – easily handling flow rates up to 1 mL/min in ESI and APCI without splitting. In the MSQ mass spectrometry detector ion source, a constant low flow of solvent is delivered to the edge of the source entrance cone to prevent buildup of involatiles during analysis. This "cone wash" eluent flow does not occlude the entrance orifice, but serves only to wash away deposited contamination. If possible, nonvolatile eluent components should be avoided, however, because residual salts can foul the inlet, leading to increased maintenance.

The self-cleaning API source allows for extended periods of operation with chromatographic buffers, such as phosphates or ion pairing agents, and samples in dirty matrices. Thus, the MSQ mass spectrometry detector greatly improves the precision of analysis without compromising the analytical method.

Figure 1-7. Magnified view of entrance cone with cone wash

	Electrospray Ionization (ESI) Thermally Assisted Pneumatic Nebulization	Atmospheric Pressure Chemical Ionization (APCI)
Summary	The sample solution is sprayed from a hollow capillary needle across a high-potential difference (a few kilovolts) into an orifice in the interface. Heat and gas flows are used to desolvate the ions existing in the sample solution. ESI can produce multiply charged ions with the number of charges tending to increase as the molecular weight increases.	A corona discharge is used to ionize the analyte in the atmospheric pressure region. The gas-phase ionization in APCI is more effective than ESI for analyzing less-polar species. ESI and APCI are complementary methods.
Sample Introduction	Flow injection	Flow injection
	LC/MS	LC/MS
	IC/MS	Typical flow rates are from 200 µL/min up to over 1 mL/min
	Typical flow rates are from 50 µL/min up to >1 mL/min	
Benefits	Good for charged, polar, or basic compounds	Good for less polar compounds
	Excellent for IC or reversed-phase LC	Excellent liquid chromatographic interface for normal phase separations
	Permits detection of high-mass compounds at low mass-to-charge ratios (*m/z* less than 2000)	Complementary to ESI
	Very low chemical background yields excellent detection limits	Can control presence or absence of fragmentation by varying the interface potentials
	Can control presence or absence of fragmentation by varying interface potentials	
Mass Range	Low-moderate. Typically less than 1000 Da (singly charged *m/z*)	Low-moderate. Typically less than 1000 Da (singly charged *m/z* only in APCI)
	Med-high. Typically less than 100,000 Da (multiply charged *m/z*)	

2. Life Sciences Applications: Biological and Pharmaceutical Compounds

Synthetic Peptide: (Tyr-1)-Somatostatin

Introduction

(Tyr-1)-somatostatin is a synthetic peptide with a molecular weight of 1730.3 g/mol. Synthetic peptides have a wide range of uses, including structure/function analysis, binding assays, and use as receptor agonist/antagonists or immunogens for production of specific antibodies. These compounds are also tested as cancer therapeutics, and their purity must be checked for quality control.

Analysis

For the separation, a Thermo Scientific™ Acclaim™ C18 (300 Å) column was used with 25% acetonitrile, 0.1% formic acid, and 74.9% water. The analyte elutes with a retention time of 1.73 min and can be detected in the UV range at 274 nm. The UV spectrum is presented in Figure 2-1.

For polar analytes such as peptides, ESI is the preferred ionization technique. The addition of 0.1% formic acid improves the formation of protonated adducts, which are detected in positive mode. Depending on the molecular weight, peptides can form multiply charged ions in ESI. Figure 2-2 shows the mass spectrum of a 30 mg/L (Tyr-1)-somatostatin reference solution. Under these conditions the triply charged quasi-molecular ion at 577.5 *m/z* is the base peak but the doubly charged quasimolecular ion at 866.2 *m/z* is also detected. Using the *m/z* of the base peak, a mass-selective chromatogram was extracted (Figure 2-3).

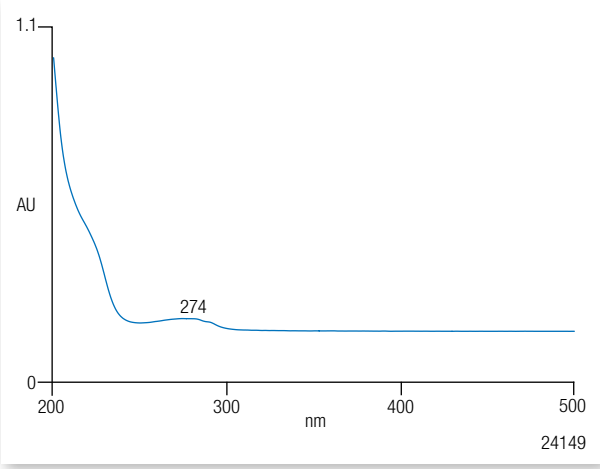


Figure 2-1. UV Spectrum of (Tyr-1)-somatostatin

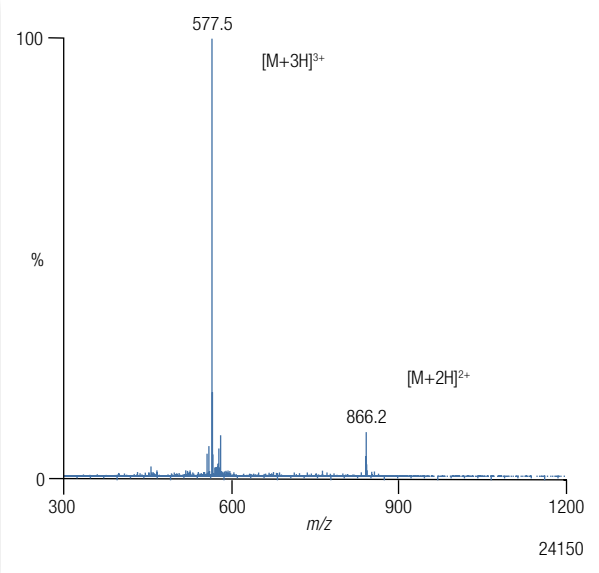


Figure 2-2. MS spectrum of (Tyr-1)-somatostatin, ESI positive, cone voltage: 75 V, scan time: 1 s, mass range: 300–2000 *m/z*

Column:	Acclaim 300, C18, 3 µm, 300 Å, 2.1 × 150 mm
Eluent:	A: Water w/ 0.1% formic acid B: Acetonitrile
Gradient:	95% A and 5% B isocratic, in 6 min to 65% B, hold for 12 min, in 0.5 min to 5% B, hold for 9.5 min
Flow Rate:	0.30 mL/min
Inj. Volume:	10 µL
Temperature:	35 °C
Detection:	MSQ, ESI+, needle voltage: 3.0 kV; probe temperature: 400 °C; cone voltage: 75 V
Peaks:	1. (Tyr-1) Somatostatin 577.5 *m/z*

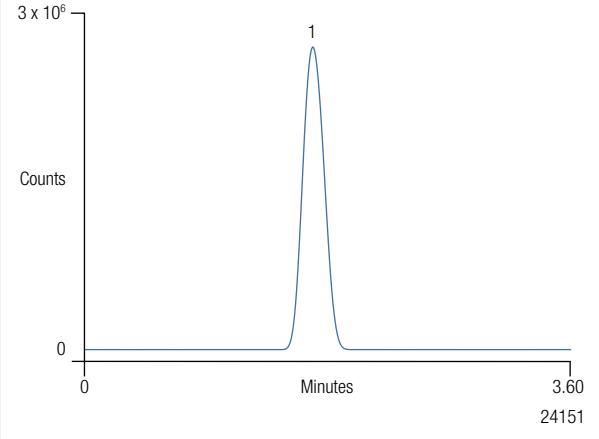


Figure 2-3. Extracted MS chromatogram of (Tyr-1)-somatostatin, ESI positive, cone voltage: 75 V, SIM: 577.6 *m/z*

Corticosteroids (Hydroxycortisone and Prednisolone)

Introduction

Hydrocortisone and prednisolone are corticosteroids (hormones produced naturally by the adrenal gland) that have many important functions, including control of inflammatory responses.

Corticosteroids work by acting within cells to prevent the release of certain chemicals which produce immune and allergic responses, resulting in inflammation. By decreasing the release of these chemicals in a particular area, inflammation is reduced. This can help control a wide number of disease states characterized by excessive inflammation, such as allergic reactions, asthma, and arthritis.

Analysis

Corticosteroids can be separated isocratically on the Acclaim C18 column using 30% acetonitrile and 70% water containing 10 mmol/L potassium hydrogen phosphate at pH 2.5. Under these conditions, prednisolone elutes at 10.8 min, and hydrocortisone at 11.5 min.

Due to the presence of phosphate buffer, the analytes form phosphate adducts which can be detected in the MS using ESI in the negative mode. Figure 2-4 shows the mass spectrum of prednisolone with cone voltage set to 40 V. The base peak is the prednisolone phosphate adduct [M+Phosphate]$^-$ at 457 *m/z*. Figure 2-5 shows the ESI mass spectrum of hydrocortisone obtained in negative mode. Hydrocortisone is also detected as the phosphate adduct [M+Phosphate]$^-$ at 459 *m/z*. The patented cone wash function was used with deionized (DI) water at a flow rate of 50 µL/min to overcome the effects of salt deposition on the entrance orifice due to the phosphate buffer. Figure 2-6 shows the overlaid selected ion monitoring (SIM) chromatograms of a mixed reference solution of 0.1 mg/L of each corticosteroid.

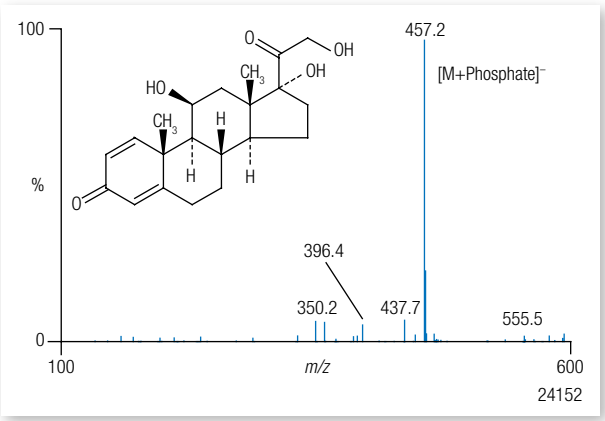


Figure 2-4. MS spectra of prednisolone phosphate adduct ESI negative, cone voltage: 40 V

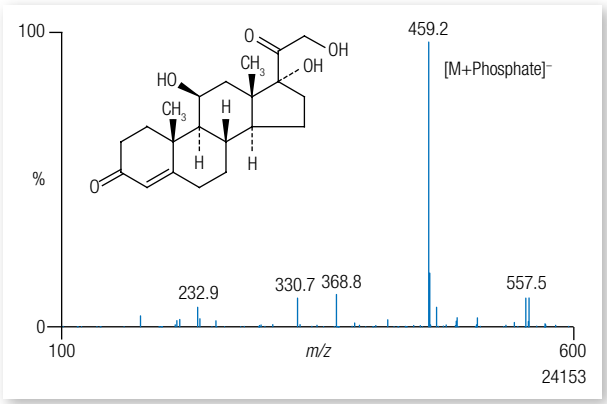


Figure 2-5. MS spectra of hydrocortisone phosphate adduct, ESI negative, cone voltage: 40 V

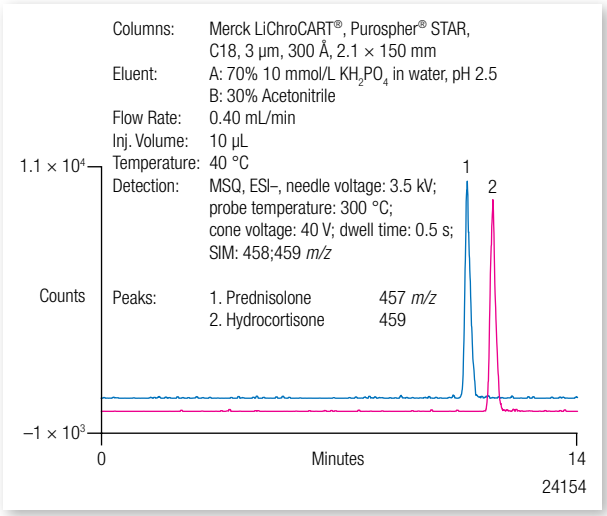


Figure 2-6. SIM chromatogram of corticosteroids as phosphate adducts

Artemisinin in an Antimalarial Drug and Blood Serum

Introduction

Artemisinin, a sesquiterpene lactone, is the key ingredient obtained from the species *Artemisia annua*, or sweet wormwood, a plant indigenous to Asia, with a long history of use as a highly effective antimalarial remedy. The compound was first isolated in 1972; investigators at the Walter Reed Army Institute of Research located and crystallized the active component in 1984.[1] However, in China, the leaves of the sweet wormwood have been used for several centuries as an antiparasitic agent and a treatment for other systemic disorders, including certain types of cancer.

Analysis

Separation was performed isocratically on an Acclaim C18 column using dodecylamine/formic acid and acetonitrile mobile phase. For MS detection, ESI was used in positive mode, applying a needle voltage of 3.0 kV and a cone voltage of 50 V. The mass spectrum of artemisinin as the dodecylamine adduct is shown in Figure 2-7. The base peak at 468 *m/z* was used for the quantitation in SIM mode. A pharmaceutical formulation (Tisane) is shown in Figure 2-8. Artemisinin elutes at 5.6 min, resulting in a total analysis time of 8 min.

A serum sample containing 30 ng of artemisinin is shown in Figure 2-9.

References

1. Klyman D. Qinghaosu (Arteminisin): An Antimalarial Drug from China, *Science* **1985**, 228 (4703): 1049–1055.

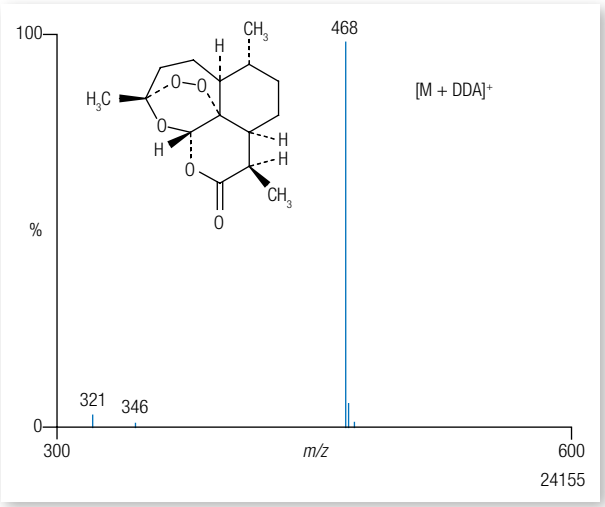


Figure 2-7. MS spectrum of artemisinin-dodecylamine adduct, ESI positive, needle voltage: 4 kV, cone voltage: 50 V

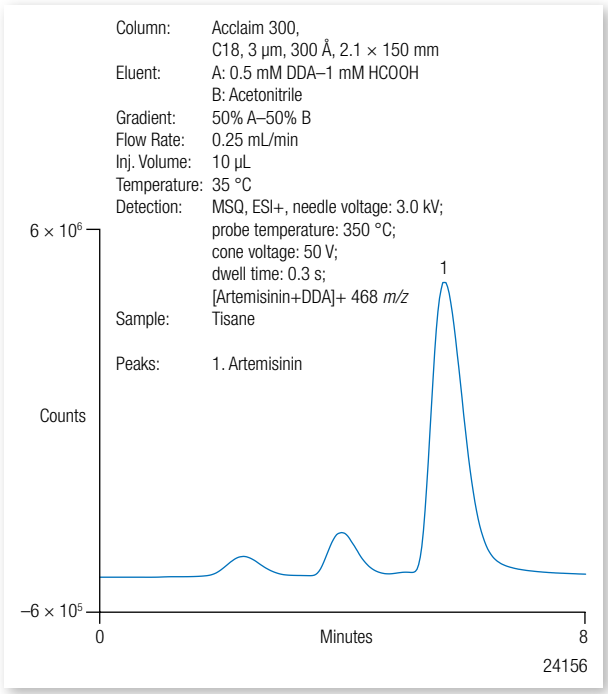


Figure 2-8. Antimalarial drug, artemisinin in pharmaceutical formulation (Tisane)

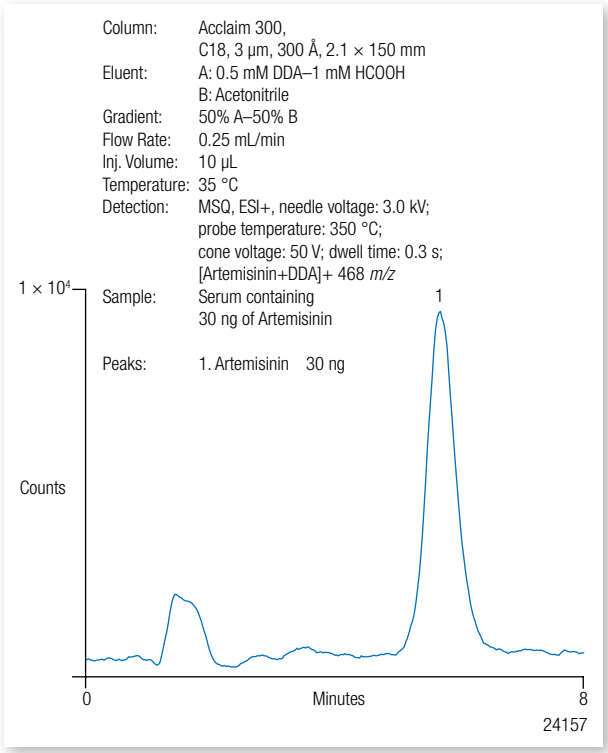


Figure 2-9. Antimalarial drug, artemisinin in serum blood sample

Diclofenac in a Pharmaceutical Formulation

Introduction

Diclofenac belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) and works by reducing levels of hormones that cause inflammation. It is used to reduce pain, inflammation, and stiffness caused by conditions such as osteoarthritis, rheumatoid arthritis, abdominal cramps associated with menstruation, and ankylosing spondylitis. Diclofenac is widely used and manufactured by several companies under different brand names, for example, Voltaren® from Sandoz Laboratories.

Analysis

Diclofenac contains two chlorine atoms, and can be detected by ESI-MS in negative ionization mode. The typical isotope pattern is observed for the quasi-molecular ion $[M-H]^-$ detected at 294, 296, and 298 *m/z*. At a cone voltage of 40 V, a significant fragment ion is also detected at 250, 252, and 254 *m/z*, showing the isotope pattern of two chlorine atoms. This fragment ion is the quasi-molecular ion with a loss of CO_2 $[M-CO_2-H]^-$. The corresponding mass spectrum is shown in Figure 2-10.

Diclofenac can be separated isocratically from other matrix components in a pharmaceutical formulation with an Acclaim 120 C18 column using 60% acetonitrile and 40% water containing 10 mmol/L ammonium formate. The separation is completed in only 8 min (Figure 2-11). Using SIM mode, a linear calibration plot (Figure 2-12) is obtained from 2 ng to 150 ng of diclofenac injected on column.

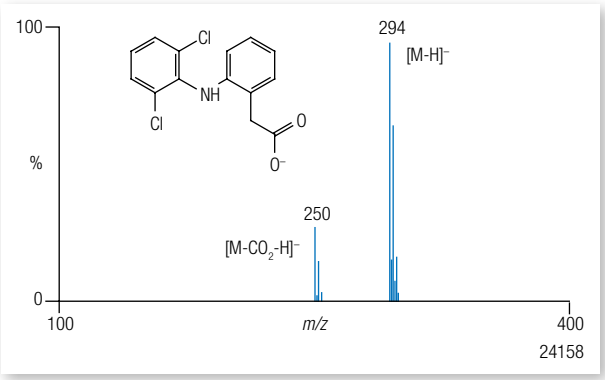


Figure 2-10. Mass spectrum of diclofenac ESI negative, needle voltage: 3.0 kV, cone voltage: 40 V

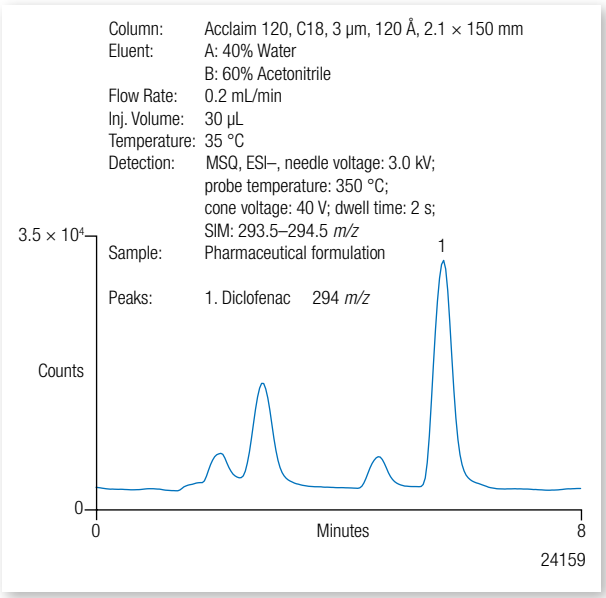


Figure 2-11. Diclofenac in a pharmaceutical formulation

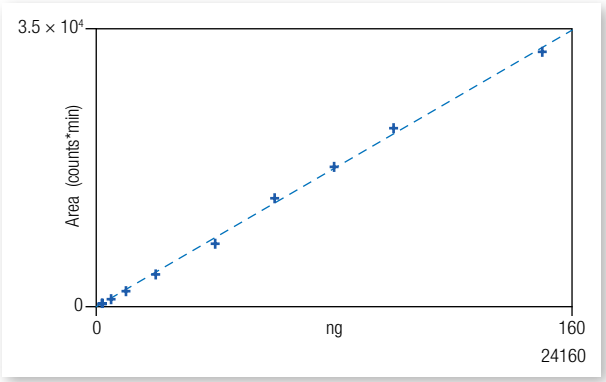


Figure 2-12. Calibration curve for diclofenac SIM 294 *m/z*, ESI negative

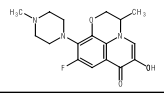
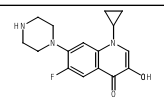
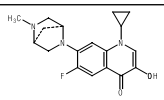
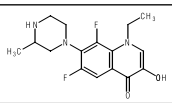
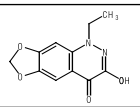
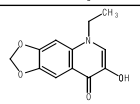
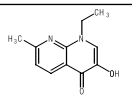
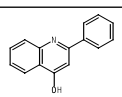
Fluoroquinolone Antibiotics

Introduction

Fluoroquinolone antibiotics are effective against a variety of bacteria, and have been widely used in both medical and veterinary applications. Use of these antibiotics in animal feed has generated growing concern because fluoroquinolone residues may promote bacterial antibiotic resistance. Efficient multiresidue methods are required to detect and measure levels of fluoroquinolones present in animal tissues and environmental samples. It is also important to confirm the identities of the fluoroquinolones detected. This application shows simultaneous measurement and confirmation of eight fluoroquinolone antibiotic compounds.

Analysis

Separation was performed on an Acclaim C18 column with a linear gradient of water/acetonitrile containing 0.15% formic acid. To obtain optimum sensitivity, the MS was operated in the SIM mode, detecting the $[M+H]^+$ ions in positive mode. Detection limits in the low µg/L range were obtained. Total run time was 17 min, including re-equilibration of the column.

Name	$[M+H]^+$ *m/z*	Structure
Ofloxacin	362	
Ciprofloxacin	332	
Danofloxacin	358	
Lomefloxacin	352	
Cinoxacin	263	
Oxolinic acid	262	
Nalidixic Acid	233	
Cinchophen	250	

24161

Figure 2-13. Eight fluoroquinolones: names, mass-to-charge ratios of the quasi-molecular ions, and structures

Column: Acclaim 120, C18, 5 µm, 120 Å, 4.6 × 250 mm
Eluent: A: Water, pH 2.5
B: 0.15% formic acid in acetonitrile
Gradient: In 10 min to 90% A and 10% B to 37.5% A and 62.5% B, then in 0.5 min to 0% A and 100% B isocratic for 2 min, in 1 min to 90% A and 10% B for 4 min
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 350 °C; cone voltage: 50 V; dwell time: 0.2 s; SIM as indicated

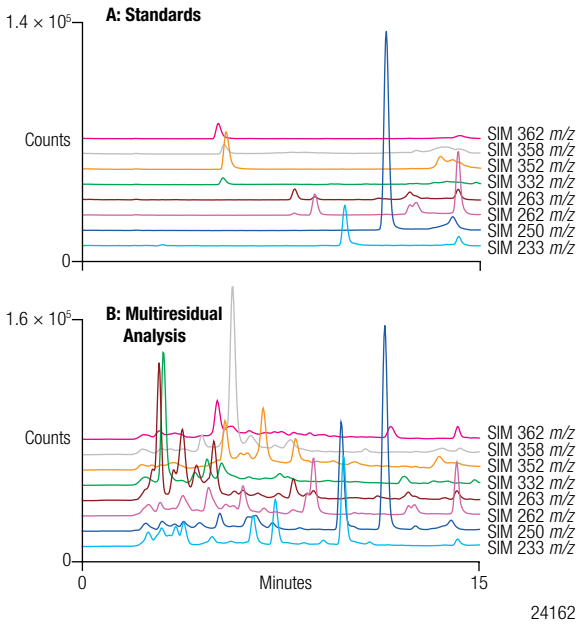


Figure 2-14. Multiresidual analysis for eight fluoroquinolones and standards

Rapamycin (Immunosuppressant) in Blood Samples

Introduction

Immunosuppressant drugs reduce a body's natural defenses against foreign invaders and therefore have a suppressive effect on the immune system. Rapamycin, also known as sirolimus, is an immunosuppressant drug used in transplant patients to help prevent rejection of transplanted organs or medical devices. It is especially useful for kidney transplant patients and those receiving coronary stents.

Scientists are currently studying the role of rapamycin and similar immunosuppressive compounds for treatment of cancer.

Analysis

Rapamycin can be determined in blood samples using 90% methanol, 10% water with 50 µmol/L sodium acetate on a reversed-phase Acclaim C18 column. MS detection is performed using ESI in the positive mode. The recorded mass spectrum is presented in Figure 2-15 showing the quasi-molecular ion $[M+Na]^+$ at 936 *m/z*.

Separation is complete in less than 10 min. A comparison of UV and MS detection is presented in Figure 2-16. A calibration plot from 5 to 40 ng is shown in Figure 2-17 with a linear curve fit. Other immunosuppressants, such as cyclosporin-A, tacrolimus, and everolimus can also be determined in whole blood samples using this technique. An Acclaim 120 Guard column was used to remove particulates and preserve the analytical column.

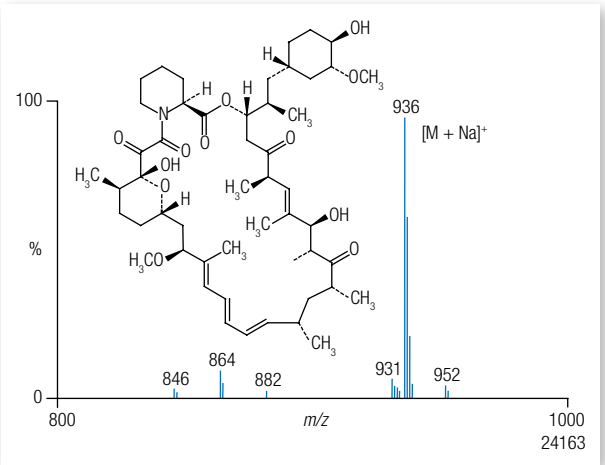


Figure 2-15. Mass spectrum of rapamycin

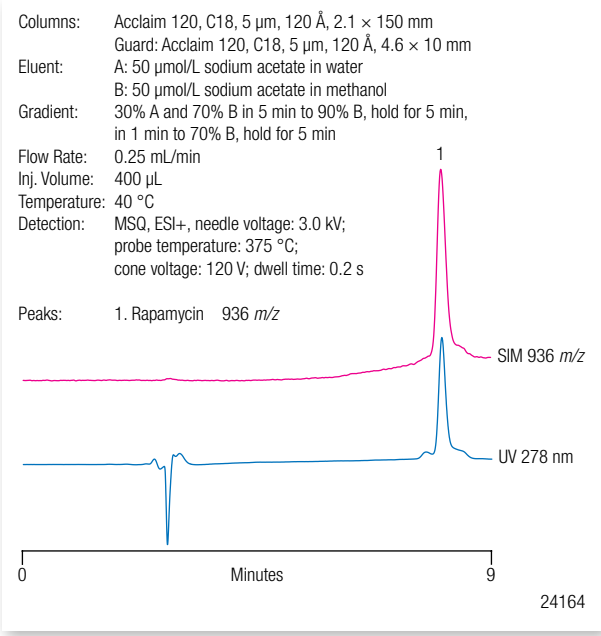


Figure 2-16. Overlay of UV and MS signals

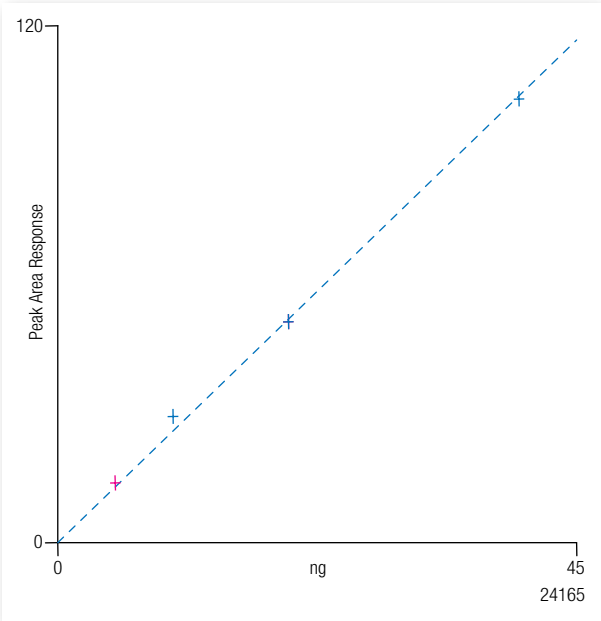


Figure 2-17. Calibration curve for rapamycin (5–40 ng, SIM 936 *m/z*)

Additives (Bacitracin and Avoparcin) in Animal Feed

Introduction

Bacitracin and avoparcin are antibiotics previously authorized in the European Union for use in animal feed. Avoparcin is also used as a growth-enhancing agent in animal nutrition for poultry, pigs, and cattle. Bacitracin is obtained from the bacterium *Bacillus licheniformis* and is composed of several peptides (A, B, C, D, E, F1-3). Bacitracin A cyclic dodecapeptide is the most dominant and important component providing up to 70% of the structure. It requires divalent cations, such as zinc, to form stable complexes in order to function effectively as a bactericide. Like other similar antibiotics, Bacitracin inhibits biosynthesis of the cell wall by binding with bactoprenyl pyrophosphate.

Avoparcin was banned in Germany in 2004. New data have confirmed that its use in animal feed promotes the development of resistance to glycopeptide antibiotics, thus reducing treatment possibilities in human medicine. For this reason, interest in analyzing these additives in animal feeds has increased.

This LC-MS method demonstrates how these antibiotics can be determined in animal feed samples. The structures of Bacitracin A and Avoparcin are shown in Figure 2-18.

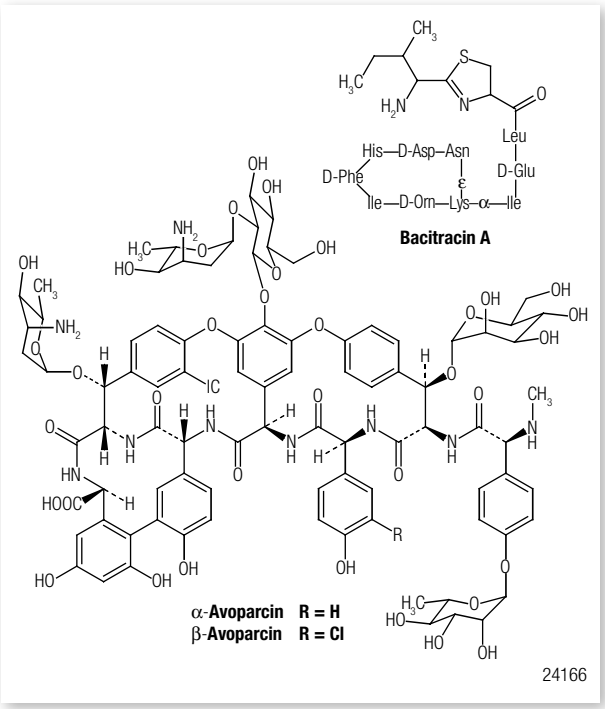


Figure 2-18. Structure of bacitracin and avoparcin

Analysis

Both additives are compounds with high molecular weights. For example, α-avoparcin has a monoisotopic mass of 1907.6 g/mol. It can be detected by ESI in the positive mode as a triply charged quasi-molecular ion $[M+3H]^{3+}$ at 637.9 *m/z*. β-Avoparcin has a molecular weight of 1941.6 g/mol and is also detected as a triply charged quasi-molecular ion $[M+3H]^{3}+$ at 648.2 *m/z*. Zn bacitracin is detected at 474.9 *m/z*.

The mass spectra of β-avoparcin and Zn bacitracin are shown in Figures 2-19 and 2-20, respectively.

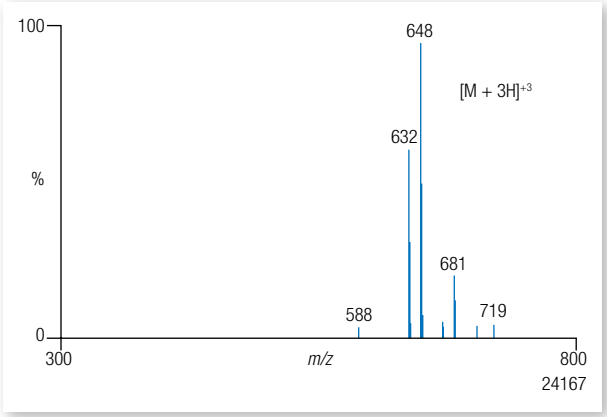


Figure 2-19. Mass spectrum β-avoparcin, MW 1943.6

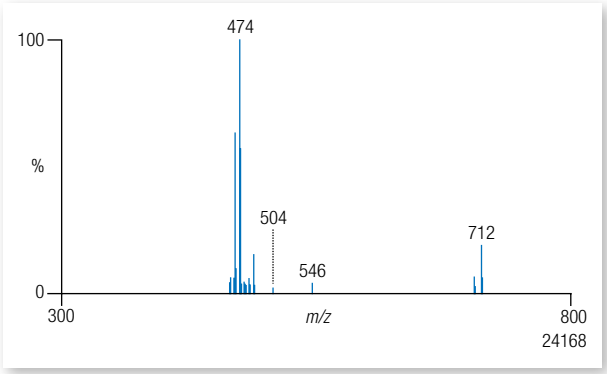


Figure 2-20. Mass spectrum of zinc bacitracin

The feed additives are extracted from feed samples and can be separated from matrix compounds by HPLC using an Acclaim C18 column with a water/acetonitrile gradient. To improve chromatographic peak shape and enhance ionization, 5 mmol formic acid is added to the mobile phase. To achieve the highest sensitivity and selectivity, the MS was operated in SIM mode.

The overlaid chromatograms of a reference solution of Zn bacitracin and avoparcin (5 mg/L each) and a feed sample extract are shown in Figure 2-21. The feed sample extract shows it contains Zn bacitracin, but no avoparcin.

Column:	Acclaim 120, C18, 3 µm, 120 Å, 2.1 × 150 mm
Eluent:	A: 5 mmol/L formic acid in water B: 5 mmol/L formic acid in acetonitrile
Gradient:	2 min 100% A and 0% B, in 10 min to 0% A and 100% B, isocratic for 1 min, then in 1 min to 100% A and 0% B isocratic for 4 min
Flow Rate:	0.4 mL/min
Inj. Volume:	10 µL
Temperature:	30 °C
Detection:	1. UV: 254 nm; 3-D: 200–600 nm 2. MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 350 °C; cone voltage: 30 V; 1 s; dwell time: 0.2 s; SIM1: 474.7–475.7 (0–18 min); SIM2: 647.9–648.9 (0–18 min)

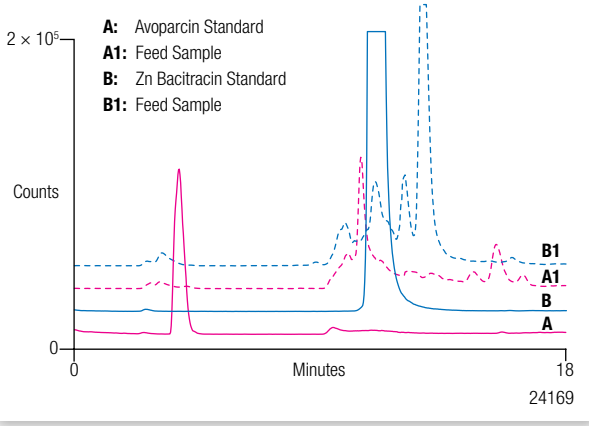


Figure 2-21. Chromatograms of reference solutions of zinc bacitracin and avoparcin in a feed sample

Tween 80 and Triton X-100 in Serum

Introduction

Tween® 80 (polyoxyethylene sorbitan esters) and Triton® X-100 (octyl phenol ethoxylate) are mixtures of compounds with different degrees of ethoxylation. Both products are used as surfactants, emulsifiers, and stabilizers in pharmaceutical products. For quantitation in complex matrices such as serum, typically one or two representative marker compounds of Tween 80 and Triton X-100 are used to simplify the analysis.

Analysis

Tween 80 and Triton X-100 are separated on an Acclaim C18 column using a mobile phase of 30% water and 70% acetonitrile. Figure 2-22 shows the chromatogram of a test solution containing 13.3 mg/L Tween 80 and 10 mg/L Triton X-100.

Both analytes display broad peaks. Tween 80 elutes first with a retention time of 2 min, followed by Triton X-100 with a retention time range of 5–9 min. MS detection was performed using ESI in positive mode, scanning from 101–1211 *m/z* in 1 s, with a cone voltage of 50 V.

The mass spectra were averaged over the entire peak. Figure 2-23 shows the mass spectrum of Tween 80. Because Tween 80 is a complex mixture, many mass peaks are detected. The peak at 650.5 *m/z* was used as a marker for later detection in the SIM.

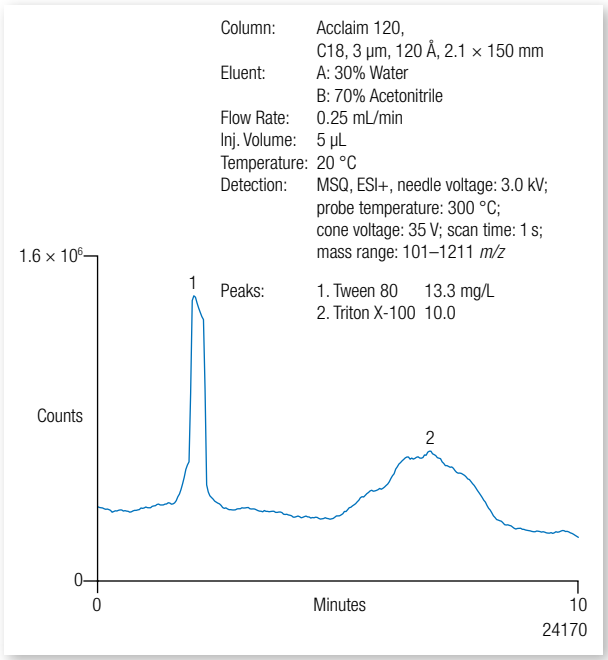


Figure 2-22. Total ion chromatogram of test solution of Tween 80 and Triton X-100

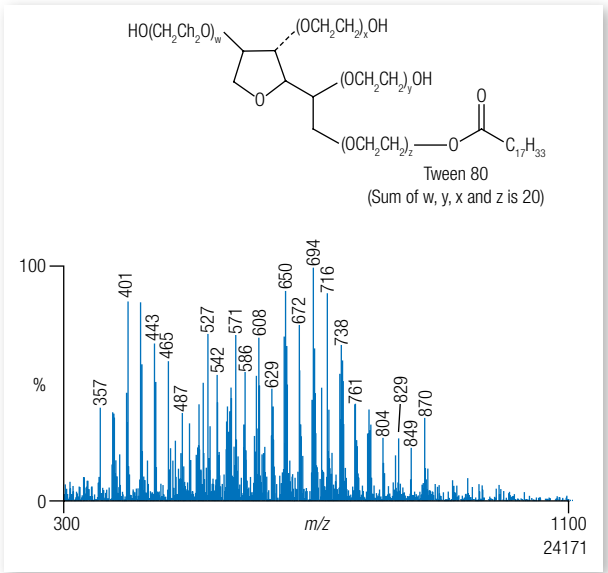


Figure 2-23. MS spectrum of Tween 80

The mass spectrum of Triton X-100 is presented in Figure 2-24. Triton X-100 is a mixture of ethoxylated octylphenols. These compounds differ by 44 atomic mass units according to their degree of ethoxylation. The base peak is detected at 576.5 *m/z*.

Figure 2-25 shows the determination of Tween 80 and Triton X-100 in blood serum. The chromatogram is the sum of the two individual SIM channels (SIM1: 650.5 *m/z* for Tween 80 and SIM2: 576.5 *m/z* for Triton X-100).

Using these marker peaks allows quantitation of both additives in blood serum samples.

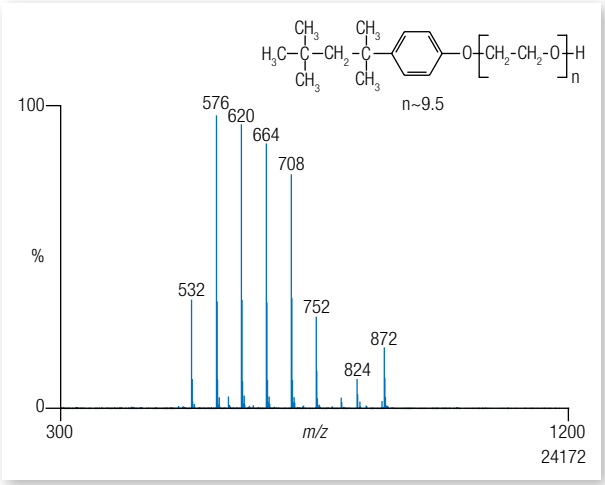


Figure 2-24. Mass spectrum of Triton X-100

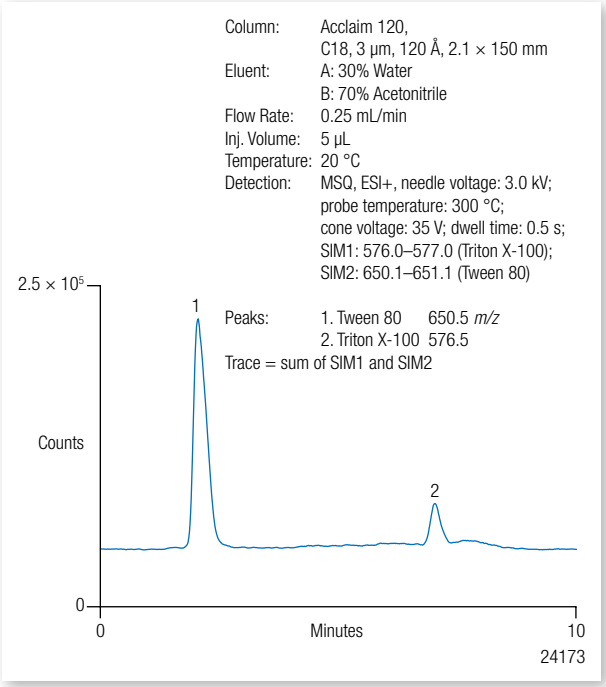


Figure 2-25. Chromatogram of Tween 80 and Triton X-100 in blood serum

3. Environmental Applications: Water Testing

Acrylamide in Water

Introduction

Acrylamide, a known neurotoxin and human carcinogen, has been found in certain prepared foods, and has recently been included among the substances to be monitored in drinking water according to the last European Community Directive on potable water. A new method for determination of this compound based on the combination of ion-exclusion chromatographic separation and MS detection has been developed accordingly. A sample of drinking water can be directly injected onto the microbore Thermo Scientific™ Dionex™ IonPac™ ICE-AS1 column and detected in SIM mode by a single quadrupole system with ESI. Chromatographic conditions, such as eluent composition and flow rate, have been optimized by a Central Composite Design (CCD) experiment. Statistical analysis shows that the acetonitrile component of the eluent mixture is the variable that most readily influences retention of the acrylamide peak. After optimization of MS detection parameters, this method was validated for analysis of drinking water samples.

Analysis

Analysis was performed on a Thermo Scientific Dionex ion chromatograph coupled with the MSQ mass spectrometry detector. A 4 × 250 mm i.d. Dionex IonPac ICE-AS1 column was used with acetonitrile/formic acid eluent.

The MS was operated in positive electrospray mode. Cone voltage was set at 50 V. The protonated molecular ion $[M+H]^+$ of acrylamide has been detected at a mass-to-charge ratio 72 *m/z*.

Results

The mass spectrum of acrylamide in total ion current mode is shown in Figure 3-1. For the quantitative measurements SIM mode at 72 *m/z* was used.

The use of SIM detection mode assures better sensitivity and selectivity for this analysis. To achieve sub µg/L limits of detection, large-volume injection (up to 1 mL) was used. Recovery and repeatability were estimated at two levels (0.5 and 1 µg/L) by consecutive 10-fold injections of the samples. Recoveries were 95.5% and 97% and CVs were 16% and 12% respectively for 0.5 and 1 µg/L spiked solutions. Limit of detection (LOD) was estimated as three times the standard deviation of the sample at the lowest concentration (0.5 µg/L) or 0.20 µg/L. A chromatogram of spiked tap water is shown in Figure 3-2.

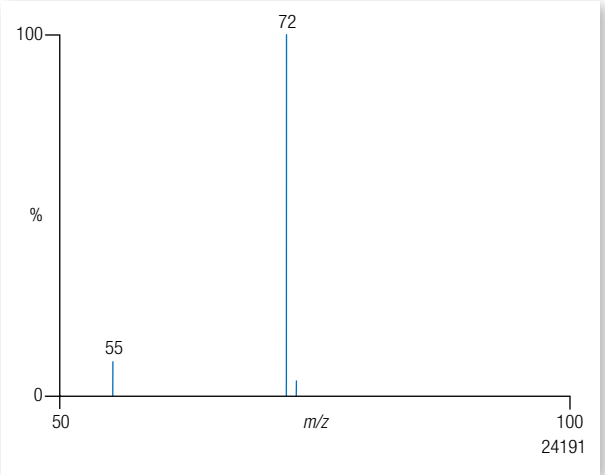


Figure 3-1. MS spectrum of acrylamide

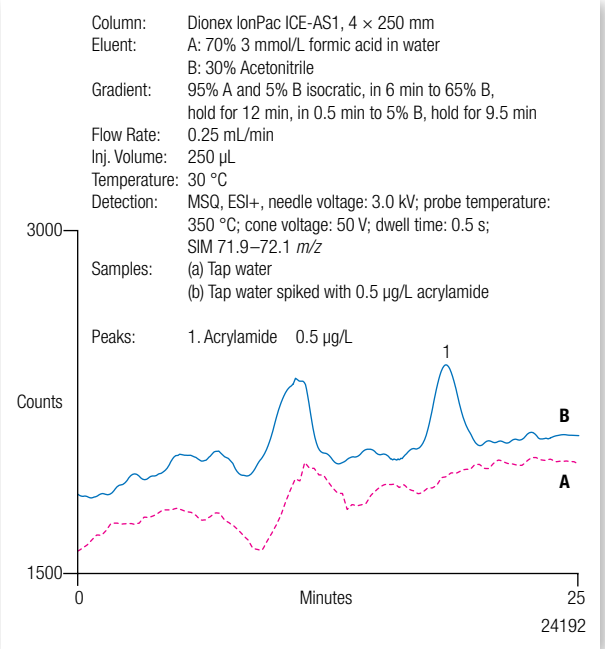


Figure 3-2. Chromatogram of spiked tap water

Triazine Pesticides in Water

Introduction

Atrazine is a selective herbicide used for the control of weeds in crops such as asparagus, corn, sorghum, sugar cane, and pineapple. It is also used in forestry for non-selective weed control in non-crop areas. Today, it is the most widely used herbicide in the United States. As a result of widespread and long-term usage, atrazine and other triazine herbicide metabolites can be found in the environment and groundwater at low levels. Monitoring of atrazine, its metabolites desethyl-atrazine and desisopropylazine, and other triazine herbicides is important for environmental protection and food safety control.

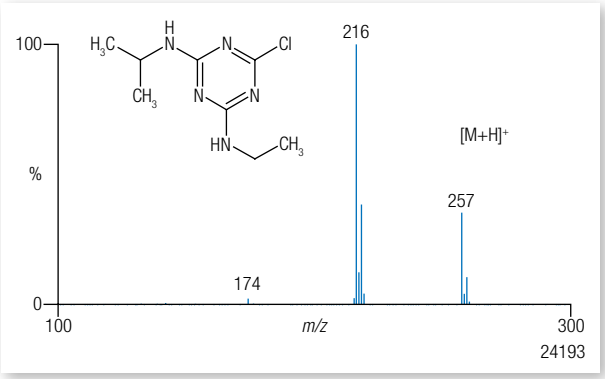


Figure 3-3. Mass spectrum of atrazine, ESI positive, cone voltage: 50 V, scan time: 1 s, mass range: 100–300 *m/z*

Column:	Acclaim 120, C18, 3 µm, 120 Å, 2.1 × 100 mm
Eluent:	A: 40 mmol/L formic acid in water B: Acetonitrile
Gradient:	95% A and 5% B isocratic, in 6 min to 65% B, hold for 12 min, in 0.5 min to 5% B, hold for 9.5 min
Flow Rate:	0.25 mL/min
Inj. Volume:	10 µL
Temperature:	30 °C
Detection:	MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 400 °C; cone voltage: 50 V; dwell time: 0.5 s

Peaks:	1. Atrazine-desisopropyl	SIM1: 173.9–174.3 (0–5 min)
	2. Atrazine-desethyl	SIM2: 187.9–188.3 (2–8 min)
	3. Simazine	SIM3: 202.0–202.4 (8–15 min)
	4. Atrazine	SIM4: 216.1–216.5 (12–18 min)
	5,6. Terbuthylazine/Propazine	SIM5: 230.1–230.5 (17–22 min)

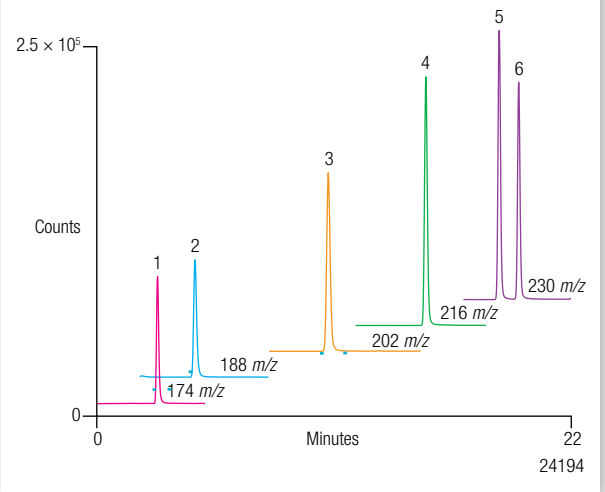


Figure 3-4. SIM chromatograms of different pesticides

Analysis

Triazine pesticides can be separated by HPLC using an Acclaim 120 C18 column and an acetonitrile/water gradient. Triazine herbicides are detected using ESI in positive mode. The mass spectrum in Figure 3-3 shows the typical isotope pattern of these chlorine-containing compounds. In addition, acetonitrile adducts are sometimes formed and detected by these compounds using these conditions. Samples were analyzed in SIM mode to achieve highest sensitivity. A typical chromatogram is presented in Figure 3-4. Detection limits were approximately 1 µg/L.

Figure 3-5 shows the lowest level chromatogram obtained in SIM mode for terbuthylazine and propazine at 1 µg/L.

Figure 3-6 shows a linear range greater than three orders of magnitude for terbutylazine and propazine.

Column:	Acclaim 120, C18, 3 µm, 120 Å, 2.1 × 100 mm
Eluent:	A: 40 mmol/L formic acid in water B: Acetonitrile
Gradient:	95% A and 5% B isocratic, in 6 min to 65% B, hold for 12 min, in 0.5 min to 5% B, hold for 9.5 min
Flow Rate:	0.25 mL/min
Inj. Volume:	10 µL
Temperature:	30 °C
Detection:	MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 400 °C; cone voltage: 50 V; dwell time: 0.5 s; SIM 230 *m/z*

Peaks:	1. Terbutylazine 1 ug/L
	2. Propazine 1

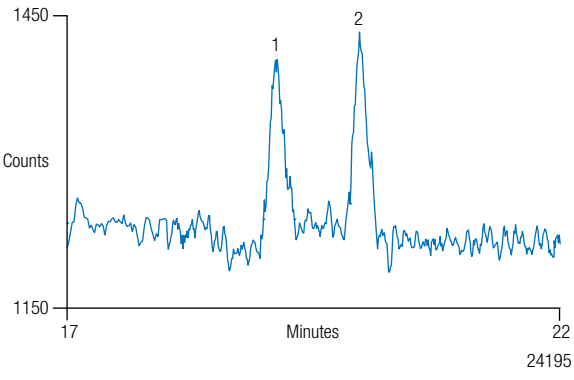


Figure 3-5. SIM chromatogram of atrazines: standard solution

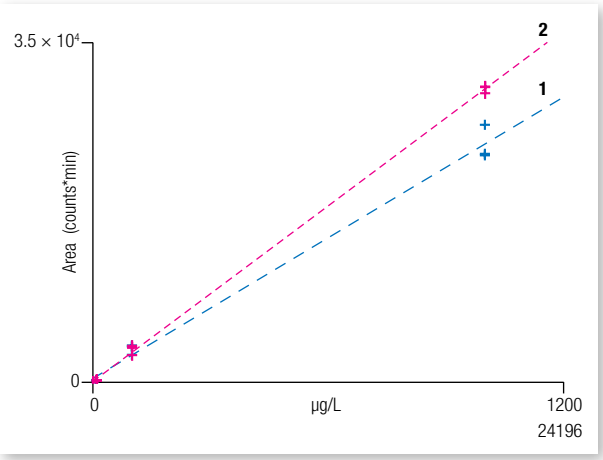


Figure 3-6. Calibration curves for (1) terbutylazine and (2) propazine

Trace Urea in Ultrapure Water

Introduction

Low levels of many organic contaminants are found in the source waters for semiconductor manufacturing plants. Urea contamination of feed water can occur from its use as a fertilizer and subsequent runoff in water tables. Organic contaminants in ultrapure water are a concern for the semiconductor manufacturing industry because they cause interference in the etching process. Parts per billion (ppb) level monitoring of urea in ultrapure water is critical to the integrity of the photolithography process as the presence of urea can alter the photochemical properties of the photoresist on the wafer. The removal of organics at the point-of-use is necessary as these are only partially removed by conventional purification technologies.

Analysis

The ultrapure water samples were analyzed in positive ESI mode by the MSQ mass spectrometry detector after elution from the ion chromatograph using 2.5 mM MSA eluent. The eluent was prepared manually by diluting 3 mL stock of 1 N MSA solution to 1 L of eluent. Working standards of urea were prepared by appropriate dilutions of a stock 1 ppm standard. Dilutions were made using the mobile phase to ensure the pH of samples.

A low dead-volume static mixing tee (P/N: 063143) was used to add 0.25 mL/min solution of acetonitrile/water (50:50) prior to the MSQ mass spectrometry detector to assist in desolvation of the eluent and in the formation of a stable electrospray. Urea was detected in the SIM mode, using the pseudo-molecular ion $(M+H)^+$ at 61 *m/z*. Conditions with the SIM trace are seen in Figure 3-7A. An example of a full-scan spectrum of the urea chromatographic peak is seen in Figure 3-7B. The spectrum also shows acetonitrile and its dimer (*m/z* 41 and 82 respectively), the pseudo-molecular ion of urea, and urea adducts with acetonitrile and MSA.

Results

One of the challenges of quantitating urea by LC-MS is retaining the analyte out of the void volume of the chromatographic system. Using the Dionex IonPac columns and with the parameters given, the analysis for urea can be accomplished in less than 5 min, with urea retained on the column 2 min out of the void.

Lower levels of detection can be reached by increasing the injected sample volume or by the additional use of sample preconcentration.

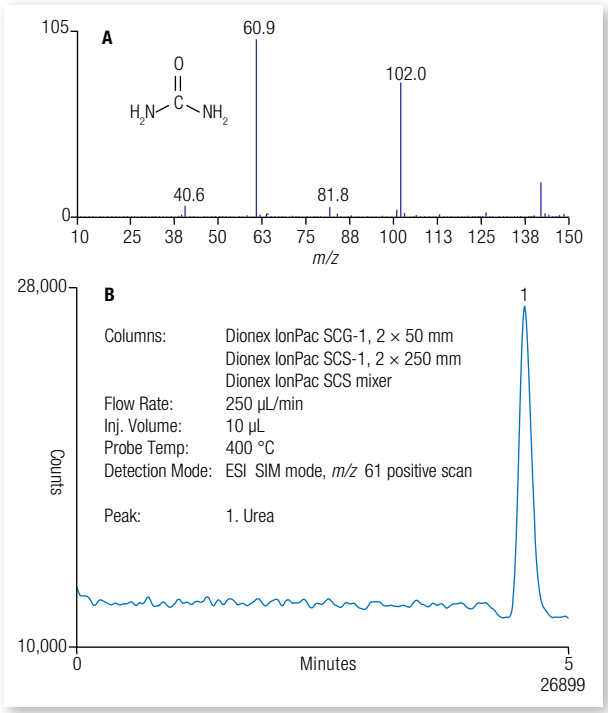


Figure 3-7. A) Full-scan spectrum and B) SIM trace of the urea chromatographic peak

Carbamates in Water

Introduction

N-methyl carbamates (NMCs) are widely used pesticides and have been reported in the environment and food. They are endocrine disruptors that exert an anticholinesterase action on the nervous system. N-methyl carbamates are relatively unstable compounds that break down in the environment fairly rapidly.

This application describes a routine LC-MS method for direct analysis of sub-ppb level NMCs in water samples without lab-intensive sample enrichment, excessive sample preparation, or derivatization.

Analysis

A standard pesticide mixture containing 10 carbamates was diluted into methanol as a working solutions to prepare calibration standards. An internal standard, carbaryl-d_7 for MS detection, was prepared by dissolving in methanol to 500 ppb. Calibration standards were prepared by series dilutions of the working solution to 20, 10, 5, 1, 0.5, and 0.2, with IStd spiked at 20 ppb at each level.

When no buffer was added in the mobile phase, NMCs were observed as protonated molecular ion ($[M+H]^+$) or adducts ($[M+NH_4]^+$). Ammonium formate buffer (0.05 mM) was added into the mobile phase to facilitate the formation of analyte-ammonium adducts to improve the sensitivity for some analytes. SIM scan parameters for each analyte are seen in Table 3-1.

Table 3-1. SIM Scan Events for Carbamates

Name	SIM	Adduct	Dwell Time (s)	Cone Voltage
Aldicarb	208	$[M+NH_4]^+$	0.3	40
Aldicarb sulfone	240	$[M+NH_4]^+$	0.2	50
Aldicarb sulfoxide	224	$[M+NH_4]^+$	0.3	35
Carbaryl	219	$[M+NH_4]^+$	0.1	35
Carbaryl-d_7 (IStd)	226	$[M+NH_4]^+$	0.1	35
Carbofuran	222	$[M+H]^+$	0.1	40
3-Hydroxycarbofuran	255	$[M+NH_4]^+$	0.3	50
Methiocarb	226	$[M+H]^+$	0.3	30
Methomyl	163	$[M+H]^+$	0.3	30
Oxamyl	237	$[M+NH_4]^+$	0.2	30
Propoxur	210	$[M+H]^+$	0.1	40

Results and Conclusions

The improved chromatographic performance is seen in Figure 3-8. Ten target analytes were separated to baseline in 18 min. The use of MS detection provides enhanced selectivity and sensitivity. Method detection limits (MDL) were achieved for all analytes at sub-ppb level, ranging from 0.017 (3-hydroxycarbofuran) to 0.059 ppb (methiocarb). Good correlation of determination ($r^2 > 0.99$) was achieved for each analyte through two orders of magnitude in the calibration range (0.2 ppb to 10 ppb).

This method was successfully applied to analyzing water samples collected from sources probably containing trace-levels of NMCs and confirmed the presence of aldicarb and carbaryl at sub-ppb levels.

System: Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system
Column: Acclaim Carbamate column, 2.1 × 150 mm, 3 µm
Mobile Phase: A) Methanol
B) 1.0 mM ammonium formate
C) Water

Time (min)	A%	B%	C%
–4.0	10	5	85
2.0	10	5	85
15.0	65	5	30
15.1	90	5	5
20.0	90	5	5

Flow Rate: 300 µL/min
Inj. Volume: 20 µL
Detector: Thermo Scientific™ MSQ Plus™ single quadrupole mass spectrometer

Mass Spectrometric Conditions
Ionization Interface: ESI positive mode
Detection Mode: SIM

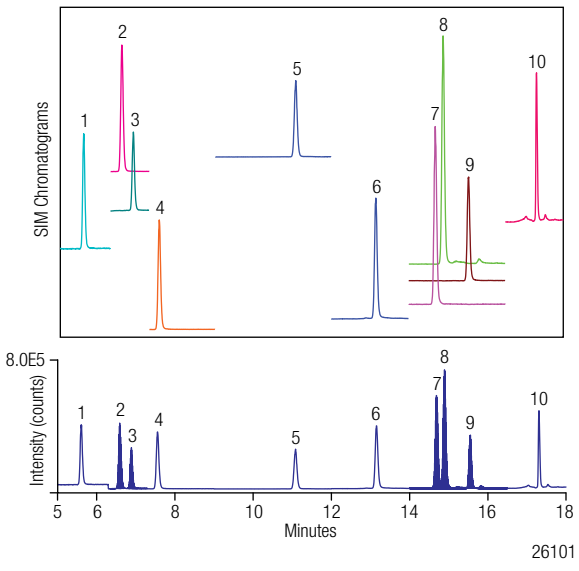


Figure 3-8. Total ion chromatogram (TIC) and SIM chromatogram of 10 baseline separated N-methyl carbamates

Nitrosamines in Drinking Water

Introduction

N-Nitrosodimethylamine and other structurally similar nitrosamines are classified as probable carcinogens by the U.S. Environmental Protection Agency (EPA), and their presence have been reported in California drinking water since 1998. Established notification levels for these nitrosamines have been set to 10 ng/L (parts per trillion).

Analysis of trace levels of nitrosamines in water samples involves solid phase extraction (SPE) and concentration, followed by liquid or gas chromatographic separation with a sensitive and selective detector, preferably mass spectrometry.

This LC-MS method demonstrates the improved chromatographic separation and detection using an MSQ Plus single quadrupole mass spectrometer.

Analysis

For this analysis, an UltiMate 3000 HPLC system was coupled to an MSQ Plus single quadrupole mass spectrometer by an ESI interface. Chromatographic separation was achieved on an Acclaim PA2 column with gradient elution composed of 0.5–80% acetonitrile from 5 to 21 min, 80% for 2 min, and 0.005% formic acid held constant throughout the run. The MSQ Plus single quadrupole mass spectrometer was operated in SIM mode, and the protonated molecular ion $[M+H]^+$ was used as the quantification ion for each of the analytes.

MS parameters, such as probe temperature, needle voltage and cone voltages, were optimized to provide the best sensitivity and are shown in Figure 3-9.

Results

As seen in Figure 3-9, the UV chromatogram shows well-resolved chromatographic separation (20 ng of each analyte in a 20 µL injection), and the MS SIM chromatograms demonstrate the advantage of MS for selective and sensitive detection (2 ng of each analyte in a 20 µL injection). For N-nitrosodiphenylamine (Figure 3-9, peak 10), interferences were chromatographically separated from the target analyte, showing the combined benefits of chromatographic separation and MS detection in achieving a high level of confidence in quantitation. With the addition of proper SPE sample enrichment, this method provides sufficient sensitivity to enable detection of nitrosamines in water samples at low parts-per-trillion levels.

System:	UltiMate 3000 HPLC system
Column:	Acclaim PA2, 2.1 × 150 mm, 2.2 µm
Gradient:	A) Acetonitrile; B) 0.1% formic acid; C) DI water 0.5% to 80% A from 5 min to 20 min, hold for 2 min; Hold B constant at 5%
Flow Rate:	250 µL/min
Injection:	20 µL
Column Temp:	10 °C

Mass Spectrometric Conditions

System:	MSQ Plus single quadrupole mass spectrometer
Interface:	ESI
Probe Temp:	450 °C
Needle Voltage:	2500 V
Nebulizer Gas:	Nitrogen at 85 psi
Detection Mode:	SIM

Analyte	t_R (min)	SIM (*m/z*)
1. N-Nitrosodimethylamine	2.9	75
2. N-Nitrosomorpholine	4.4	117
3. N-Nitrosomethylethylamine	4.9	89
4. N-Nitrosopyrrolidine	5.2	101
5. N-Nitrosodiethylamine	9.9	103
6. N-Nitrosopiperidine	11.1	115
7. N-Nitrosodipropylamine-d_{14}	15.8	145
8. N-Nitrosodipropylamine	16.1	131
9. N-Nitrosodibutylamine	19.5	159
10. N-Nitrosodiphenylamine	20.5	199

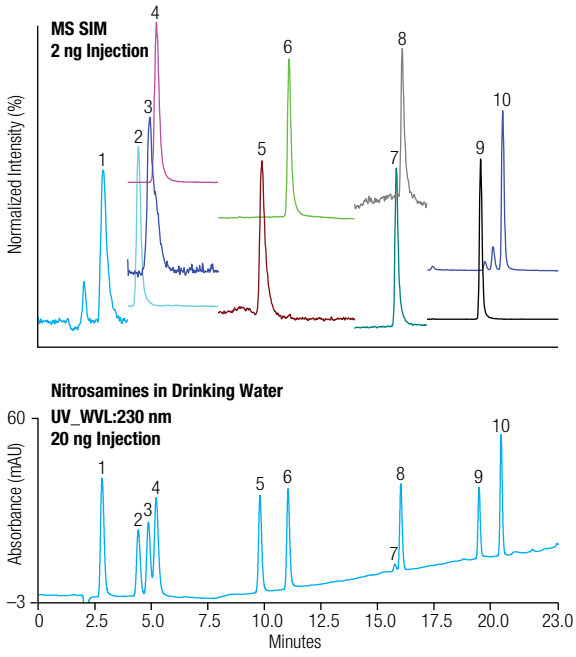


Figure 3-9. N-Nitrosodimethylamine and other nitrosamines by LC-MS

Common Cations and Amines

Introduction

IC has been extensively used as the preferred separation technique for ionic species such as inorganic anions/cations, small amines and organic acids, peptides and proteins, nucleic acids, and carbohydrates. In recent years, the increasing demands for higher sensitivity, selectivity, confirmative information, and structural information leads to the emergence of MS as a powerful complementary detector to conductivity, UV, and electrochemical detection.

This method demonstrates the use of IC with MS detection for the determination of six commonly seen cations and selected amines. Confirmative information was achieved using full-scan MS spectra showing positively charged cation species and characteristic adduct patterns. Quantification was achieved using SIM acquisitions for each specific analyte.

Analysis

The experiment was performed on a Thermo Scientific Dionex ICS-2000 Reagent-Free™ IC (RFIC™) system with separation of target analytes achieved on a Dionex IonPac CS12A-5 µm MSA selective column. The column was operated at 30 °C with 0.5 mL/min flow rate. An eluent generator with MSA cartridge was used to generate 33 mM isocratic MSA for the elution and separation of target analytes.

The MSQ Plus single quadrupole mass spectrometer was coupled to the IC via an ESI interface, and operated in SIM mode. The external water was delivered at 1 mL/min as the regenerant for the continuous regeneration of suppressor. Isopropanol was delivered by a Thermo Scientific Dionex AXP-MS pump at 0.2 mL/min to improve the desolvation/ionization efficiency.

Results

As seen in Figure 3-10, six cations were chromatographically separated and detected by the MSQ Plus single quadrupole mass spectrometer with excellent selectivity and sensitivity. The studied amines were observed co-eluting with cations. However, each of these amines was observed as a single peak in monitored SIM channels, demonstrating the great selectivity offered by mass spectrometric detection.

With mass spectrometric detection, confirmation of the analyte identity can be achieved from full-scan spectra, and quantitation can be achieved at low ppb levels with greater confidence by using selective SIM scans.

System: Dionex ICS-2000 RFIC ion chromatography system
Column: Dionex IonPac CS12A-5 µm, 3 × 150 mm with guard column, 3 × 50 mm, and a continuously regenerated cation trap column CR-CTC, 2 mm
Mobile Phase: Isocratic 33 mM MSA, electrolytically generated
Flow Rate: 0.5 mL/min
Injection Volume: 100 µL
Temperature: 30 °C
Detection: Suppressed conductivity, external water mode
MSQ Plus mass spectrometry detector

Mass Spectrometric Conditions
System: MSQ Plus single quadrupole mass spectrometer
Interface: ESI
Solvent: 0.2 mL/min isopropanol delivered by a Dionex AXP-MS auxiliary pump
Detection Mode: Positive SIM
Needle Voltage: 2 kV
Nebulizer Gas: Nitrogen at 85 psi
Probe Temp.: 500 °C

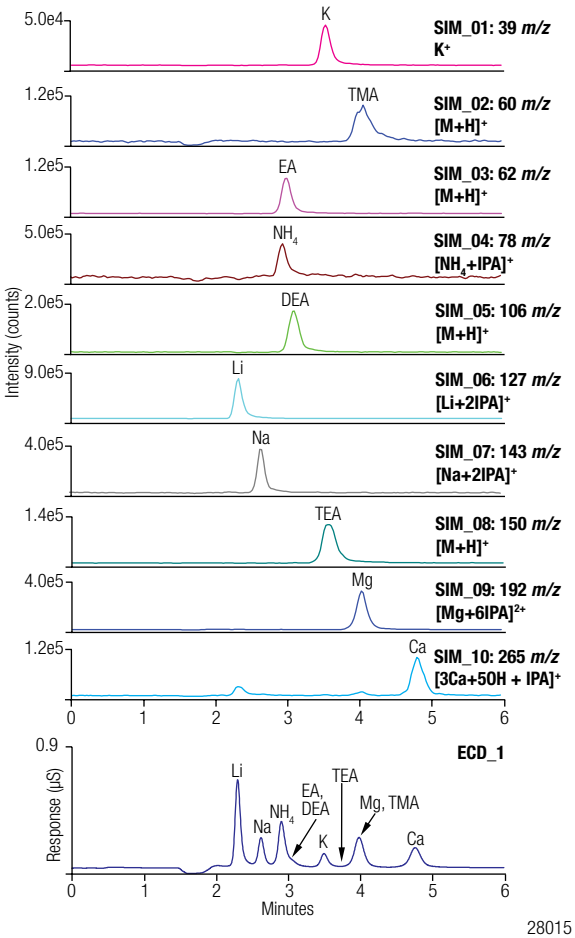


Figure 3-10. SIM and conductivity chromatograms of six cations and four amines

Common Anions and Organic Acids

Introduction

MS as a detector for IC has gained popularity recently due to increasing demands for sensitivity, selectivity, confirmation of identity, and structural interpretation. Compared to other commercially available MS detectors, the MSQ Plus mass spectrometry detector offers substantially improved performance for low molecular weight analytes, and addresses most analytes for small molecule IC applications. This study demonstrates the ion chromatography mass spectrometry ion chromatography mass spectrometry (IC-MS) method using the MSQ Plus mass spectrometry detector for the determination of five common anions and selected organic acids.

Analysis

The experiment was performed on a Dionex ICS-2000 RFIC system with separation of target analytes achieved on a Dionex IonPac AS20 hydroxide selective column. The column was operated at 30 °C with 0.25 mL/min flow rate. An eluent generator with KOH cartridge was used to generate 28 mM isocratic hydroxide for the elution and separation of target analytes.

The MSQ Plus single quadrupole mass spectrometer was coupled to the IC via an ESI interface, and operated in SIM mode. The external water was delivered at 0.5 mL/min as the regenerant for the continuous regeneration of suppressor. Acetonitrile was delivered by a Dionex AXP-MS pump at 0.22 mL/min to improve the desolvation/ionization efficiency.

Results

As seen in Figure 3-11, target analytes were chromatographically separated and detected by the MSQ Plus single quadrupole mass spectrometer with excellent selectivity and sensitivity. The addition of the MS detector for quantitative analysis not only significantly improves the method sensitivity, but also provides the selectivity to differentiate analyte by mass-to-charge ratio. When operated in full-scan mode for qualitative analysis, the mass spectrometric detector provides spectra for identity confirmation and structure interpretation (with the presence of fragment ions).

System:	Dionex ICS-2000 RFIC system
Columns:	Dionex IonPac AS20, 2.1 × 250 mm Dionex IonPac AG20, 2.1 × 50 mm Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column
Mobile Phase:	28 mM hydroxide generated from EGC II KOH cartridge
Flow Rate:	0.25 mL/min
Injection:	5 µL
Detection:	Suppressed conductivity, external water at 0.50 mL/min

Mass Spectrometric Conditions

System:	MSQ Plus single quadrupole mass spectrometer
Interface:	ESI
Solvent:	0.22 mL/min acetonitrile delivered by a Dionex AXP-MS auxiliary pump
Detection Mode:	Negative SIM
Needle Voltage:	1.5 kV
Nebulizer Gas:	Nitrogen at 85 psi
Probe Temp:	450 °C

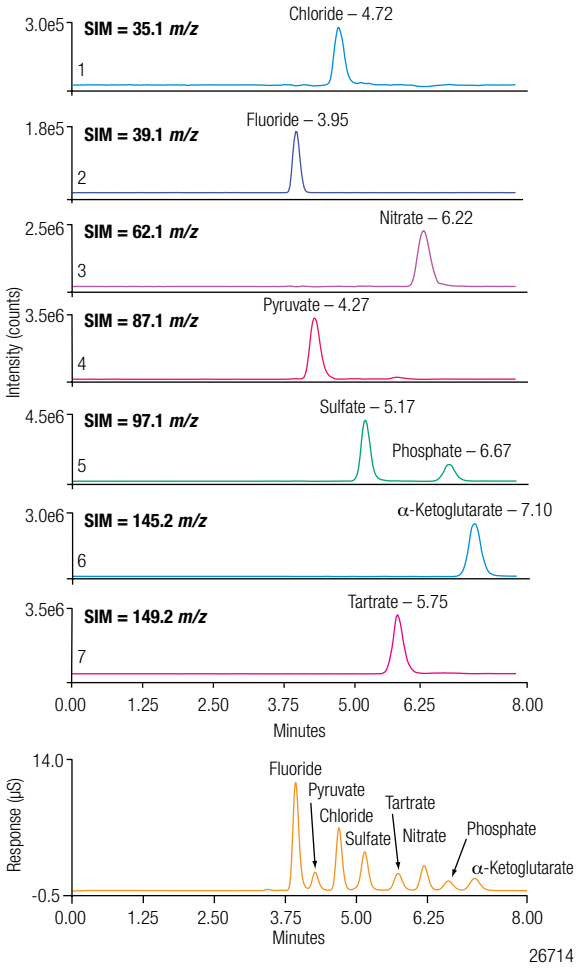


Figure 3-11. Conductivity and SIM chromatograms of five common anions and three selected organic acids

Endothall in Water Samples

Introduction

Endothall is a widely used herbicide for both terrestrial and aquatic weeds. Exposure to endothall in excess of the maximum contamination level (MCL) can cause illness. Exposure to high concentrations for a short period of time can cause hemorrhaging of the gastrointestinal tract, while longer-term exposure can also affect the liver and kidney. Endothall is regulated by the U.S. Environmental Protection Agency (EPA) with an MCL at 100 ppb in drinking water, and by the California EPA at 0.58 mg/L or 580 ppb as the public health goal (PHG). Current analytical methods for quantitation of endothall in water samples described in EPA method 548.1 involves time-consuming sample preparation and derivatization followed by a 20 min analysis by GC-MS or GC-FID.

This study describes the direct analysis of trace levels of endothall in water samples by IC-MS. Water samples were directly injected for analysis and chromatographic separation was only 10 min. The MSQ Plus single quadrupole mass spectrometer was operated in SIM mode allowing minimum sample cleanup and ensuring sensitive (low ppb) and selective quantification. Isotope labeled glutaric acid (Glutarate–d_6) was used as the internal standard to ensure quantitation accuracy.

Analysis

Analyses were performed using a Thermo Scientific Dionex ICS-5000 RFIC system and a hydroxide selective Dionex IonPac AS16 column with a temperature of 30 ºC and a flow rate of 0.4 mL/min.

A hydroxide gradient from an eluent generation KOH cartridge was used for the elution and separation of target analytes. The gradient started with 15 mM hydroxide after a 4 min equilibration, ramped up to 80 mM from 5 to 6 min and held for 3 min, and then returned to initial condition in 0.5 min. Suppressed conductivity and the MSQ Plus single quadrupole mass spectrometer were used as detectors in series.

The MSQ Plus single quadrupole mass spectrometer was coupled to the IC via an ESI interface and operated in SIM mode. IC eluent was directed to the MSQ Plus single quadrupole mass spectrometer for detection from 4.2 to 6 min, but was otherwise diverted to waste. See Figure 3-12 for SIM scan details.

Results

As seen in Figure 3-12, endothall was retained and separated from seven commonly seen anions within 10 min, and was detected with great sensitivity and selectivity using SIM acquisition. This method features direct analysis without sample pretreatment and a significant reduction in run time for improved throughput relative to GC methods. Sufficient sensitivity was achieved in this study to allow routine quantification of endothall below the lowest regulated level (100 ppb by U.S. EPA standards).

System: Dionex ICS-5000 RFIC system
Column: Dionex IonPac AS16, AG16, 2 mm
Mobile Phase: Hydroxide gradient generated from EGC II KOH cartridge

Time (min)	Conc (mM)
–4.0	15
0.0	15
5.0	15
6.0	80
9.0	80
9.5	15
10.0	15

Flow Rate: 400 µL/min
Temperature: 30 ºC
Detection: Suppressed conductivity
MSQ Plus single quadrupole mass spectrometer

Mass Spectrometric Conditions
System: MSQ Plus single quadrupole mass spectrometer
Interface: ESI
Probe Temp: 500 ºC
Needle Voltage: 3000 V
Solvent: 200 µL/min acetonitrile delivered by a Dionex AXP-MS pump
Matrix Diversion: Eluent to MS: 4.2 to 6.0 min
Detection Mode: SIM

Analyte	SIM (*m/z*)
Endothall	185 at 50 V
IS (glutarate-d_6)	137 at 35 V

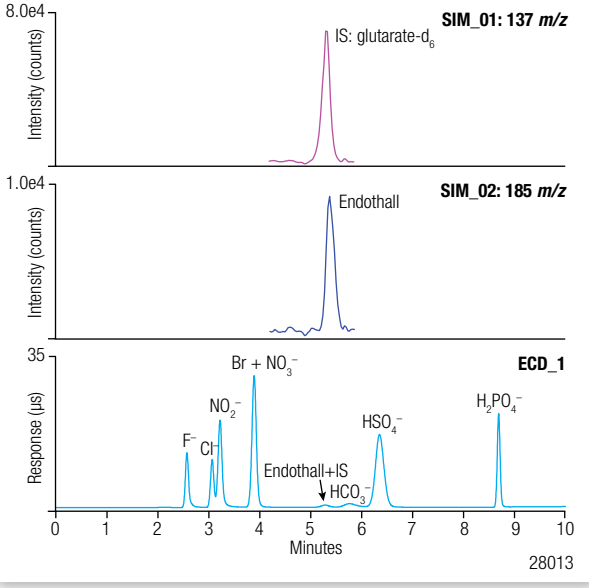


Figure 3-12. Analysis of endothall in water samples by IC-MS. 20 ppb endothall spiked in seven anions solution (0.2–1.5 ppm)

4. Environmental Applications: Health and Safety

Degradation Products of Alkylphenol Ethoxylates (Endocrine Disruptors)

Introduction

Alkylphenol polyethoxylates (APEOs) with ethoxylate units $(EO)_n$, n = 9 ~ 40 are among the most commonly used non-ionic surfactants, with an annual global production of over 650,000 tons. This consists of approximately 80% nonylphenol ethoxylates (NPEOs) and 20% octylphenol ethoxylates (OPEOs). Numerous studies have revealed the wide prevalence of APEO degradation products with known estrogenic potency and persistency (n ≤ 3, OPEO1, OPEO2, and OPEO3; NPEO1, NPEO2, and NPEO3) in water, sediments, sewage sludge, and food. Meanwhile, degradation products with different EO chains (n = 0 ~ 3) show different estrogenic potencies. APs and APEOs are regulated by many agencies, including the European Commission and the U.S. EPA. It is crucial to quantitatively determine the profile and distribution of APEO degradation products.

Analysis

A Thermo Scientific Dionex HPLC system consisting of a dual-ternary pump, autosampler, column compartment (equipped with a 2-position 10-port valve), and UVD340U detector was coupled to an MSQ Plus single quadrupole mass spectrometer by an ESI interface. A gradient mobile phase consisting of CH_3OH, ammonium acetate (NH_4OAc, 100 mM), and water was used to achieve the separation. Ammonium acetate was kept constant at 3% through the run to maintain 3 mM buffer concentration. The CH_3OH gradient and equilibration times are seen in Figure 4-1. Flow rate was kept constant at 0.4 mL/min. The MSQ Plus single quadrupole mass spectrometer was operated in a mixed-scan mode of SIM and full scan.

IGEPAL CA-210 and IGEPAL CO-210 were used as standard mixtures for OPEOs and NPEOs respectively. Each standard mixture was diluted to 0.1% (1000 ppm total for all oligomers) in CH_3CN/H_2O (50/50, v/v) as a standard stocking solution.

Column: Acclaim Surfactant
Dimensions: 2.1 × 150 mm
Mobile Phase: (A) CH_3CN
(B) NH_4OAc 100 mM pH 5.2
(C) H_2O

Time/min	%A	%B	%C
–5.0	66	3	31
8.5	66	3	31
9.5	86	3	11
16.0	86	3	11
17.0	66	3	31

Flow Rate: 0.40 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: UV, 225 nm
Sample: OPEOs 2 ppm
NPEOs 2 ppm
OP, t-OP 2 ppm

Mass Spectrometric Conditions
Ionization Interface: ESI
Detection Mode: SIM
Probe Temperature: 350 °C
Needle Voltage: 3000 V
Nebulizer Gas: Nitrogen at 80 psi

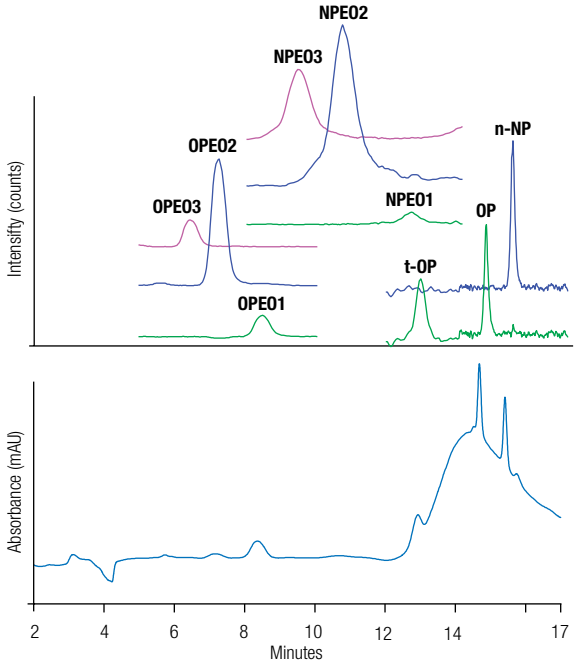


Figure 4-1. UV and SIM chromatogram overlays of alkylphenols and their mono- to triethoxylates

Results

The retention of APEO oligomers on the surfactant column follows the reverse order of EO number. APEOs with more EO units eluted from the column earlier, thus making this column a good candidate for APEO degradation products analysis. In addition, this column shows a balanced resolution for APEO oligomers and oligomer groups. Longer chain APEOs could be easily eliminated or specifically retained by adjusting the mobile phase composition.

Chromatography was optimized based on CH_3OH as organic solvent in mobile phase with NH_4OAC buffer. A gradient program was applied to elute phenols that are more polar than APEOs and retain longer on the surfactant column. UV and SIM chromatogram overlays of alkylphenols and their mono- to triethoxylates are seen in Figure 4-1.

Figure 4-2 shows the calibration curves for n-nonylphenol, OPEO2, and NPEO2. Quantification limits were estimated to be 0.05 to 2.4 ng/injection for APEOs and 5 ng/injection for APs.

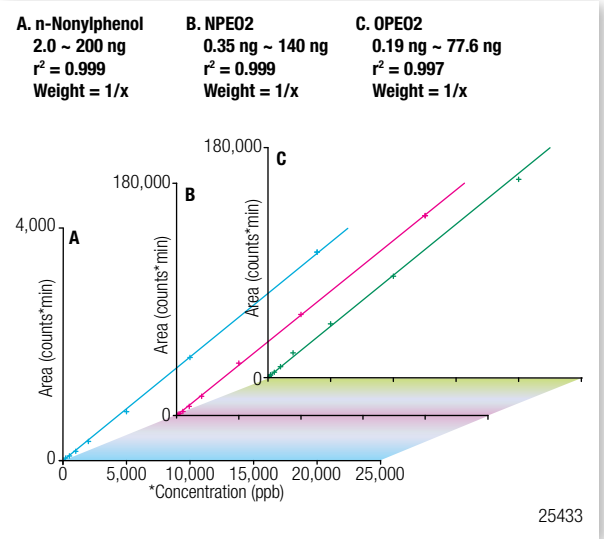


Figure 4-2. Calibration curves for n-nonylphenol, OPEO2, and NPEO2

Phenolic Acids

Introduction

Phenolic acids are aromatic secondary metabolites and serve various roles in plant life, varying from structural to protection. Researchers study phenolic acids for food quality because they are associated with color, sensory qualities, nutritional, and antioxidant properties. Recently, phenolic acids have generated significant interest due to their antioxidant properties and potential health implications. They have also been used clinically as biomarkers for organic acid disorders. The mixed-mode WAX-1 column is used to improve the retention and resolution of these phenolic acids and MS sensitivity was improved by using a mildly acidic mobile phase.

Analysis

All phenolic acids were obtained in either pure organic or sodium salt form. Each standard was weighed and dissolved in methanol/water (50/50, v/v) as individual standard stock solutions. The 15 stock solutions were then mixed in acetonitrile to 50 ppm as a primary standard mixture. A 1 to 10 dilution of the primary standard mixture in mobile phase was used as a working solution for each analyte. Calibration standards were prepared by series dilutions from the primary standard mixture to 11 levels: 2000, 1000, 500, 200, 100, 50, 20, 10, 5, 2, and 1 ppb.

Results

Chromatographic separation was carefully controlled to resolve most phenolic acids, especially phenolic acid isomers, such as *p*-, *m*- and *o*-coumaric acids and *p*-hydroxybenzoic acid and salicylic acid. Once coupled to the mass spectrometer by an ESI interface, phenolic acids predominantly form deprotonated ions $[M-H]^-$ in negative ionization mode and these were used for quantification. Optimized cone voltage ranged from 40 to 70 V for the compounds in this study, and 60 V was selected for every phenolic acid for method simplicity.

Figure 4-3 shows the UV and SIM chromatograms of 15 phenolic acids. Figure 4-4 shows the calibration curve for 2-methylhippuric acid from 1.25 ng to 175 ng injected amount. Analytical response was evaluated for each phenolic acid for over two orders of magnitudes with $r^2 > 0.995$. Method detection limits range from below 10 ppb for most of the analytes to 124 ppb (benzoic acid). This method has successfully been applied for quantitative analysis of phenolic acids found in different types of beverage and food products.

Column:	Acclaim Mixed-Mode WAX-1, 5 µm, 2.1 × 150 mm
Eluent:	A) Methanol B) 400 mM NH_4OOCH/HCOOH, pH 4 C) DI water
Gradient:	See table
Flow Rate:	0.5 mL/min
Inj. Volume:	5 µL
Temperature:	50 °C
Detection:	Top: MS Bottom: UV at 230 nm

Mass Spectrometric Conditions

Ionization Interface:	ESI
Detection Mode:	SIM
Probe Temperature:	500 °C
Needle Voltage:	1500 V
Cone Voltage:	Optimized for each SIM channel
Span:	0.5 *m/z*
Dwell Time:	0.4 s for each SIM
Nebulizer Gas:	Nitrogen at 80 psi

Gradient Program

Time (min)	A (%)	B (%)	C (%)
–10	82	5	13
1	82	5	13
2	90	5	5
8	90	5	5
10	90	10	0
21	90	10	0
22	82	5	13

Analyte	R.T.	SIM
1. 4-hydroxybenzoic acid	3.0	–137
2. 4-hydroxyphenylacetic acid	4.1	197.1
3. phenylacetic acid	4.4	181.1
4. benzoic acid	4.9	N/A
5. 3-hydroxyphenylacetic acid	5.3	325.2
6. 3-hydroxybenzoic acid	5.6	–137
7. 2-methylhippuric acid	7.0	119.1
8. 3,5-dihydroxybenzoic acid	8.3	180
9. hippuric acid	7.4	–153
10. 2-hydroxyphenylacetic acid	8.8	–151
11. homogentisic acid	10.4	–167
12. 4-hydroxyphenyllactic acid	12.7	–181.1
13. 2-hydroxyhippuric acid	13.1	–194.1
14. salicylic acid	16.5	–137
15. gentisic acid	18.3	–153

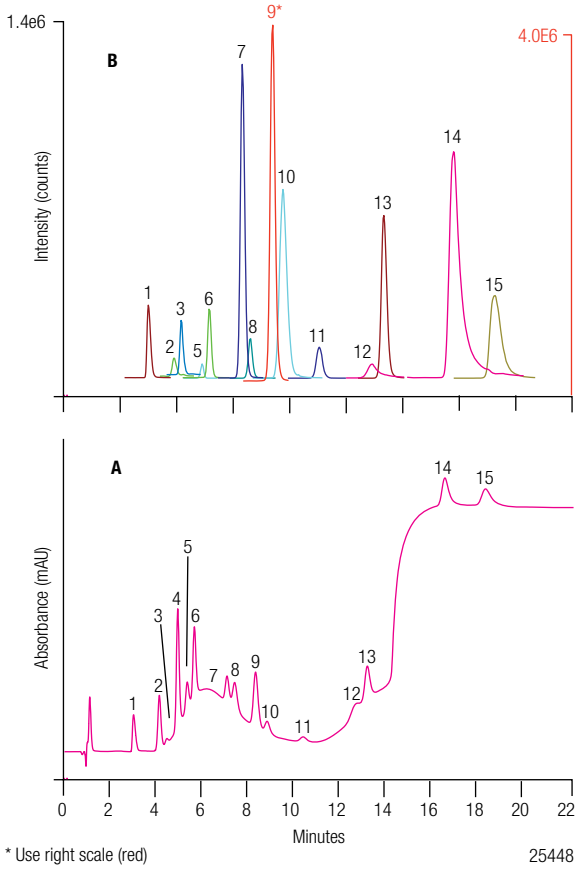


Figure 4-3. UV and SIM chromatograms of 15 phenolic acids

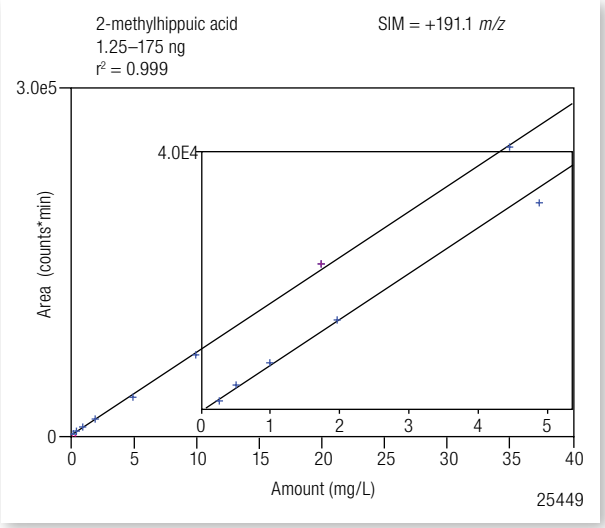


Figure 4-4. Calibration curve for 2-methylhippuric acid from 1.25 ng to 175 ng injected amount

Fluoroacetic and Trifluoroacetic Acids

Introduction

Phenolic acids are aromatic secondary metabolites and serve various Fluoroacetic acid (FA) is a naturally occurring toxic component found in poisonous plants and is used as a rodenticide. It is also an intermediate metabolite of many compounds, such as anticancer drugs 5-fluorouracil and fluoroethyl nitrosourea. Trifluoroacetic acid (TFA) is the persistent atmospheric degradation product of hydrofluorocarbons (HFCs), which is increasingly used as an alternative to ozone-damaging and banned chlorofluorocarbons (CFCs) and is known to contribute to environmental toxicity. TFA is also widely used as an HPLC mobile phase modifier in the pharmaceutical and biotechnology purification processes. It is crucial to develop a method to monitor FA and TFA for environmental risk assessment and also for their removal from products intended for human use.

Analysis

The IC-MS method is used to separate FA and TFA from other common anions based on RFIC technology, with sensitive and selective mass spectrometric detection. The chromatography and MS detection parameters are provided in the chromatogram.

Results

As seen in Figure 4-5, FA and TFA are well separated from common anions to minimize charge competition and ionization suppression. The deprotonated molecular ions ($[FA-H]^-$ at –77 *m/z*, $[TFA-H]^-$ at –113 *m/z*) were observed as the dominant species in full-scan MS spectra, and these ions were used for detection in SIM mode for quantification. Figure 4-6 shows the suppressed conductivity and MS SIM chromatograms of FA and TFA spiked at 10 ppb in an urban drinking water sample. Method detection limits (MDL) were estimated at 0.77 and 1.53 ppb for FA and TFA respectively, by replicate injections of a 10 ppb standard solution (n = 7, %RSD = 2.78% and 5.62% for FA and TFA respectively).

Column:	Dionex IonPac AG18, AS18, 2 mm
Eluent:	KOH gradient from EGC II KOH cartridge
Gradient:	Time (min) Concentration (mM) –7.0 15 0.0 15 12.0 55 12.01 75 15.0 75
Flow Rate:	0.25 mL/min
Inj. Volume:	25 µL
Temperature:	20 °C
Detection:	A: Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor™, external water mode B and C: MS

Mass Spectrometric Conditions	
Ionization Interface:	ESI
Detection Mode:	SIM
Desolvation Solvent:	Acetonitrile at 0.1 mL/min, Dionex AXP-MS pump
Probe Temperature:	450 °C
Needle Voltage:	3000 V
Cone Voltage:	45 V for FA 40 V for TFA
Nebulizer Gas:	Nitrogen at 80 psi
Scan Mode:	Negative SIM

Peaks:
1. Fluoride
2. Chloride
3. Nitrite
4. Carbonate
5. Bromide
6. Sulfate
7. Nitrate
8. Phosphate

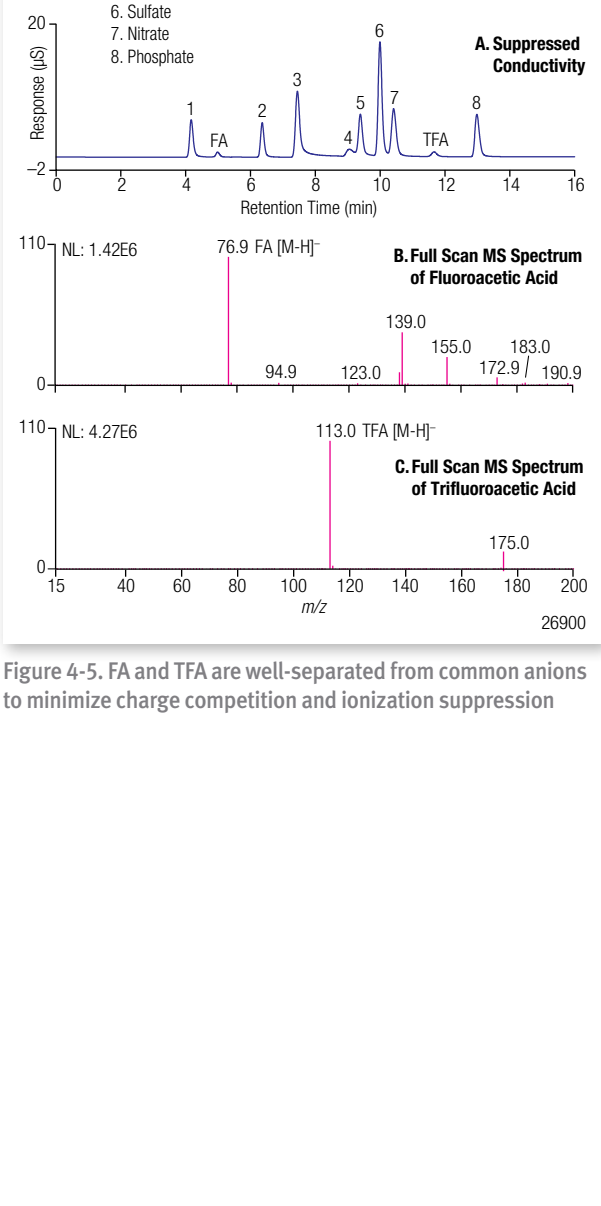


Figure 4-5. FA and TFA are well-separated from common anions to minimize charge competition and ionization suppression

Column:	Dionex IonPac AG18, AS18, 2 mm	Mass Spectrometric Conditions	
Eluent:	KOH gradient from EGC II KOH cartridge	Ionization Interface:	ESI
Gradient:	Time (min) Concentration (mM)	Detection Mode:	SIM
	–7.0 15	Desolvation Solvent:	Acetonitrile at 0.1 mL/min, Dionex AXP-MS pump
	0.0 15		
	12.0 55	Probe Temperature:	450 °C
	12.01 75	Needle Voltage:	3000 V
	15.0 75	Cone Voltage:	45 V for FA
Flow Rate:	0.25 mL/min		40 V for TFA
Inj. Volume:	25 µL	Nebulizer Gas:	Nitrogen at 80 psi
Temperature:	20 °C	Scan Mode:	Negative SIM
Detection:	A and B: Suppressed conductivity, Dionex ASRS 300 suppressor, external water mode C: MS		

Peaks:
1. Fluoride
2. Chloride
3. Nitrite
4. Carbonate
5. Sulfate
6. Nitrate
7. Phosphate

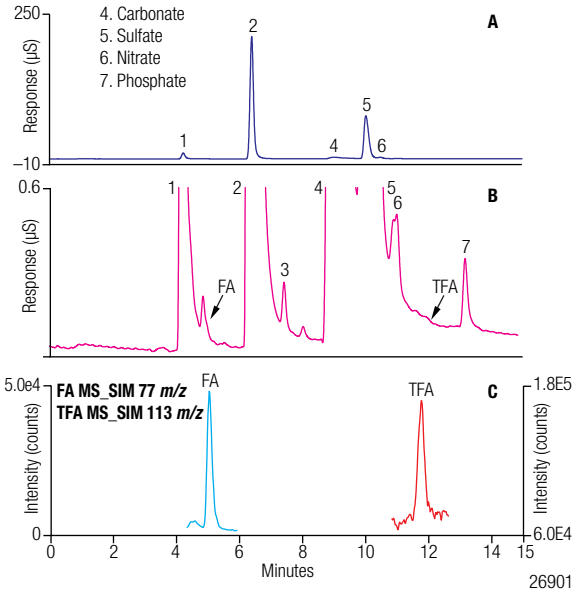


Figure 4-6. Suppressed conductivity and MS SIM chromatograms of FA and TFA spiked at 10 ppb in an urban drinking water sample

Nicotine and Metabolites

Introduction

Environmental tobacco smoke (ETS) is generated from sidestream and mainstream smoke exhaled by smokers. The relationship between ETS and numerous diseases and mortality is well-documented. Nicotine has been used as a marker for ETS exposure-assessment for decades. Its major metabolites, cotinine and trans-3'-hydroxycotinine (HOC), are used as biomarkers to study individual ETS exposure and tobacco-related diseases.

Analysis

The LC-MS was performed on a Thermo Scientific Dionex HPLC system with the MSQ Plus single quadrupole mass spectrometer operating in SIM mode. The column was an Acclaim Mixed-Mode HILIC-1 (2.1 × 150, 5 µm) with an acetonitrile/ammonium formate buffer (pH 2.7) at a flow rate of 0.25 mL/min in isocratic mode. The LC and MS parameters are as shown.

Results

The incorporated hydrophilic and reversed-phase retentions of the HILIC-1 column offer considerable retention and resolution for cotinine and HOC without sacrificing peak shape, retention, and resolution for nicotine. Figure 4-7 shows the SIM chromatograms of nicotine and metabolites, with an isotope-labeled analogue as internal standard.

Figure 4-8 shows the calibration curves of the three analytes. Excellent linearity was achieved for the three analytes within <10 ng/mL to 2000 ng/mL, with correlation coefficient (r^2) >0.999. Precision, accuracy, and method detection limits (MDL) were evaluated by replicate injections (n = 15) of medium level calibration standard. Results are shown in Table 4-1. MDLs were calculated based on the equation:

$$MDL = t_{99\%} \times S_{(n=15)}$$

where t is Student's t at 99% confidence intervals ($t_{99\%,\ n=15} = 2.624$) and S is the standard deviation. The signal-to-noise ratio of the analyte at lowest calibration level is also shown in Table 4-1.

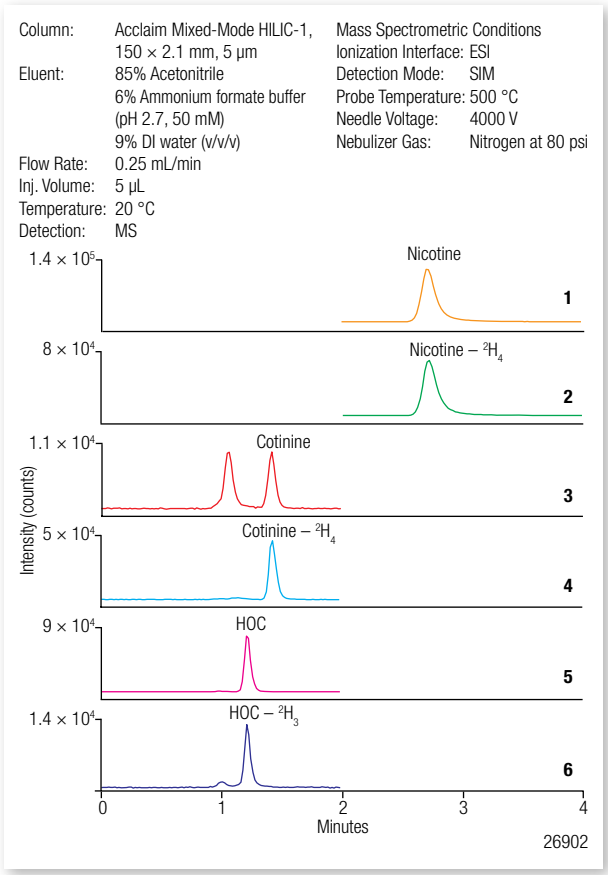


Figure 4-7. SIM chromatograms of nicotine and metabolites, with isotope-labeled analogues as internal standards

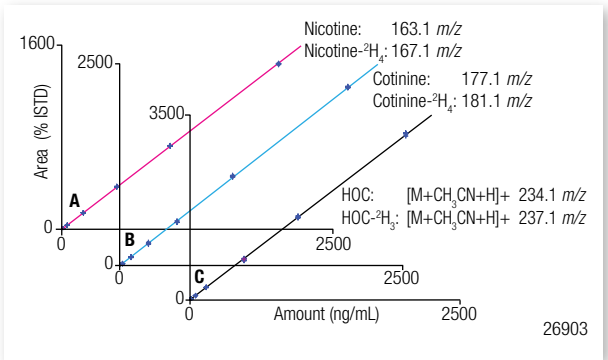


Figure 4-8. Calibration curves of the three analytes

Table 4-1. Precision, Accuracy, and Method Detection Limits (MDL)

Analyte	Expected Amount ng/mL	Observed Amount ng/mL	Standard Deviation	RSD %	Accuracy %	MDL pg	Signal-to-noise Ratio (Injected Amount)
Nicotine	199.9	200.3	0.446	0.22	100.2	5.85	25.9 (25.0 pg)
Cotinine	100.8	99.25	1.76	1.78	98.46	23.2	26.8 (10.0 pg)
3OH-Cotinine	150.0	150.6	2.25	1.49	100.4	29.5	18.7 (25.0 pg)

Glyphosates

Introduction

Glyphosate [*N*-(phosphonomethyl) glycine] is a nonselective herbicide which inhibits the shikimic acid pathway in plants. It is the most commonly used agricultural pesticide and second-most commonly used pesticide in homes and gardens. It is used to control woody and herbaceous weeds in forestry, cropped, and non-cropped sites. Although the bacteria in soil break down glyphosate into aminomethyl phosphonic acid (AMPA), wastewater discharge samples and drinking water samples in the United States and Europe have tested positive for glyphosate. Studies have raised global health and environmental concerns about the usage of glyphosate.

Analysis

Analysis of glyphosate and its breakdown product AMPA was performed on a Dionex ICS-3000 RFIC system using Dionex IonPac AG24/AS24 columns in a simulated matrix with high concentrations of chloride, carbonate, nitrate, and sulfate (250 ppm chloride and sulfate, 150 ppm sodium bicarbonate, and 20 ppm nitrate). Regions of high matrix concentrations were diverted from the source of the mass spectrometer to minimize maintenance due to source fouling. Separation on the high-capacity Dionex IonPac AS24 column provided excellent resolution of AMPA and glyphosate from the high-ionic strength matrix, while maintaining the peak shape of AMPA. Detection was by suppressed conductivity and MS SIM mode linked in series. The parameters of the separation are presented in Figure 4-9.

Results

Calibration curves generated on the MSQ Plus mass spectrometry detector show excellent linearity using external quantitative measurements, without internal standard correction. The MSQ Plus mass spectrometry detector yielded a detection range of 0.75–100 ppb for AMPA, and 0.50–100 ppb for glyphosate with r^2 values of 0.9999 for both compounds.

The method detection limit (MDL) in matrix was calculated by seven replicate injections of 10 ppb in simulated matrix. Using the equation:

$$MDL = t_{99\%} \times S_{(n-1)}$$

where t is the Student's t test at 99% confidence intervals ($t_{99\%,\ n=6} = 3.143$) and S is the standard deviation, the MDLs for both compounds were calculated. For the MSQ Plus mass spectrometry detector, the estimated MDL for AMPA in the matrix was 0.640 ppb, and the MDL for glyphosate in matrix was 0.820 ppb.

The reproducibility of 10 injections of 10 ppb standard spiked into the laboratory-simulated matrix was 5.05% for AMPA and 3.36% for glyphosate.

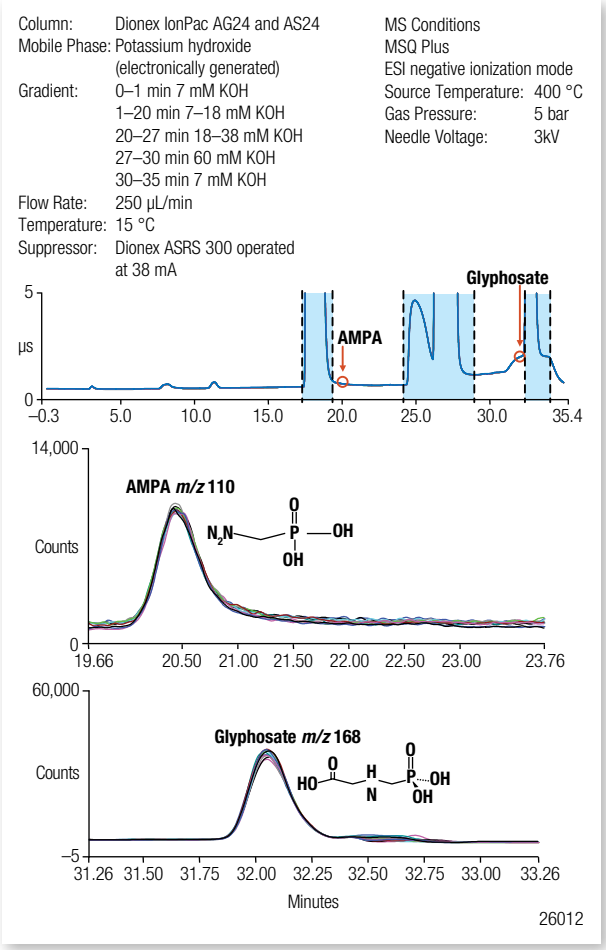


Figure 4-9. Analysis of glyphosate and its breakdown product AMPA

Ethanolamines in Water Samples

Introduction

Ethanolamines have been used as bio- and environmental markers to measure potential exposure to nitrogen mustards because direct quantification of nitrogen mustard is difficult due to their instability. Ethanolamines are manufactured in large volume (over half a million tons per year) for a wide range of both industrial and domestic uses such as the manufacture of pesticides, emulsifying agents and detergents, and bactericides and cosmetics. To monitor the removal of ethanolamines from industrial discharged waste water and the extent of human and environmental exposure to nitrogen mustards, a quantitative analytical method is desired.

An LC-MS method is described here using a mixed-mode column featuring reversed-phase and cation-exchange retention mechanisms providing sufficient retention and resolution for all analytes in 6 min. The MSQ Plus single quadrupole mass spectrometer was operated in SIM mode to ensure sensitive and selective sub-ppb level detection.

Analysis

This study was performed on an UltiMate 3000 HPLC System. The separation was achieved on an Thermo Scientific™ Acclaim™ Trinity™ P1 column (2.1 × 100 mm, 3 µm) operated at 20 °C. An isocratic mobile phase was used for the separation: 90% CH_3CN, 7% DI water, and 3% ammonium formate buffer (pH 3.7, 100 mM) with a flow rate of 0.5 mL/min. A 20 µL aliquot of each sample was injected for analysis.

An ESI source was used to couple the LC-MS system, with the needle voltage set at 1000 V and probe temperature set at 500 °C. Nitrogen was used as the nebulizer gas at 80 psi. The MSQ Plus single quadrupole mass spectrometer was operated in positive SIM mode with optimized collision voltages for each of the analyte *m/z* values: ethanolamine (EA): 62 *m/z*, 30 V; diethanolamine (DEA): 106 *m/z*, 40 V; diethanolamine-d_8 (DEA-IS): 114 *m/z*, 40 V; N-methyldiethanolamine (MDEA): 120 *m/z*, 30 V; N-ethyldiethanolamine (EDEA): 134 *m/z*, 35 V; triethanolamine (TEA): 150 *m/z*, 45 V.

Results

As seen in Figure 4-10, the five target ethanolamines were retained and separated on the Acclaim Trinity column within 5 min, and selectively detected using SIM scans. Sub-ppb level analytes can be routinely quantified using this method with Figure 4-10 showing the chromatograms of a standard containing 1 ppb of each analyte. Excellent coefficient of determination was achieved with r^2 greater than 0.99 for each analyte from detection limit to 100 ppb. Detection limits were estimated as the lowest concentration of calibration standards showing a signal-to-noise ratio greater than 5 (1 ppb for ethanolamine, and 0.1 ppb for all other target analytes).

System: UltiMate 3000 HPLC system
Column: Acclaim Trinity P1, 2.1 × 100 mm, 3 µm
Mobile Phase: 90% CH_3CN; 7% DI water;
3% buffer (100 mM ammonium formate, pH 3.7)
Flow Rate: 0.5 mL/min
Injection Volume: 20 µL
Temperature: 20 °C
Standards: 1 ppb each

Mass Spectrometric Conditions
System: MSQ Plus single quadrupole mass spectrometer
Ionization Interface: ESI
Probe Temperature: 500 °C
Needle Voltage: 1kV
Nebulizer Gas: Nitrogen at 80 psi
Detection Mode: Positive SIM

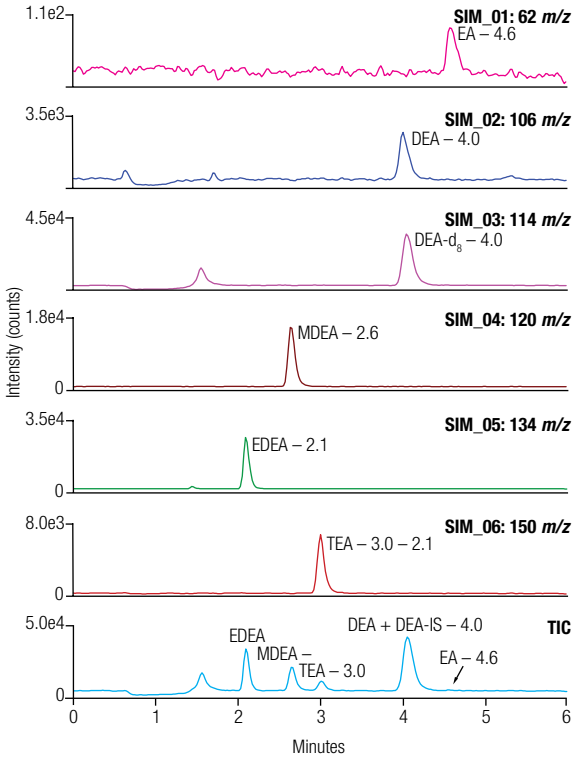


Figure 4-10. Ethanolamines (1.0 ppb each) by LC-MS

Bisphenol A, Octylphenol, and Nonylphenol in Drinking Water

Introduction

Bisphenol A (BPA) is widely used in high production volume manufacturing of polycarbonate plastics (e.g., food containers and plastic bottles) and epoxy resins. Current studies evaluating the impact of BPA on health and the environment have not reported conclusive results, however, actions have been taken proactively to protect sensitive populations. Canada proposed banning BPA to reduce the exposure for newborns and infants in 2009, and the U.S. Environmental Protection Agency (EPA) recently added BPA to its list of target chemicals for possible regulation. Octylphenol (OP) and nonylphenol (NP) are the degradation products of widely used nonionic surfactants: alkylphenol ethoxylates. The estrogenic and toxic effects of OP and NP have been well studied and reported. NP has been regulated as reported in the EPA's *Aquatic Life Ambient Water Criteria: Nonylphenol*, citing 6.6 µg/L as the maximum average level for fresh water. Recent reports show NP being detected in food and food wraps, posing more concerns for food safety and food packaging.

Here we describe a high-throughput LC-MS method for simultaneous quantitative analysis of BPA, 4-*t*-OP, 4-*n*-OP, and 4-*n*-NP.

Analysis

Separation was performed on an UltiMate 3000 HPLC system with an Acclaim PA2 RSLC column (2.1 × 50 mm, 2.2 µm) operated at 30 ºC with a flow rate of 0.5 mL/min. A gradient mobile phase was used containing methanol and water as follows: 75% to 95% methanol from 0.1 to 1.3 min; hold for 1.1 min; return to initial condition and equilibrate for 1 min.

The MSQ Plus single quadrupole mass spectrometer was interfaced to the Acclaim RSLC column by an APCI source and operated in negative SIM mode: BPA (227 *m/z*); BPA-IS (241 *m/z*); 4-*t*-OP (205 *m/z*); 4-*n*-OP (205 *m/z*); 4-*n*-NP (219 *m/z*). Probe temperature was set at 500 ºC, and corona current was set at 50 µA.

Results

BPA, 4-*t*-OP, 4-*n*-OP, and 4-*n*-NP were retained and chromatographically separated within 2.5 min. Potential interferences were also separated chromatographically, as seen in SIM_01 and SIM_02 channels. Sensitive and selective quantification was achieved at low ppb level using the MSQ Plus single quadrupole mass spectrometer. Figure 4-11 shows SIM detection at 1 ppb for BPA, and 2 ppb for the phenols spiked in a bottled water sample.

Analysis of municipal drinking water and seven randomly selected bottled water samples showed no detectable target analytes. Spiked sample analysis showed excellent recoveries: 99.2% for 4-*n*-NP, 95.6% for BPA, 76% for 4-*t*-OP, and 100.7% for 4-*n*-OP.

System: UltiMate 3000 HPLC
Column: Acclaim PA2 RSLC column, 2.1 × 50 mm, 2.2 µm
Mobile Phase: Methanol and DI water gradient:
75% to 95% methanol from 0.1 to 1.3 min, hold for 1.1 min and return to initial condition, and equilibrate for 1 min
Flow Rate: 0.5 mL/min
Inj. Volume: 100 µL
Temperature: 30 °C
Analyte: BPA 1 ppb and phenols 2 ppb in bottled water sample

Mass Spectrometric Conditions
System: MSQ Plus single quadrupole mass spectrometer
Interface: APCI
Probe Temp: 500 °C
Nebulizer Gas: Nitrogen at 55 psi
Corona Current: 50 µA
Detection Mode: Negative SIM
Cone voltage set at 65 V for all SIM scans
BPA: 227 *m/z*
BPA-IS: 241 *m/z*
4-*t*-OP: 205 *m/z*
4-*n*-OP: 205 *m/z*
4-*n*-NP: 219 *m/z*

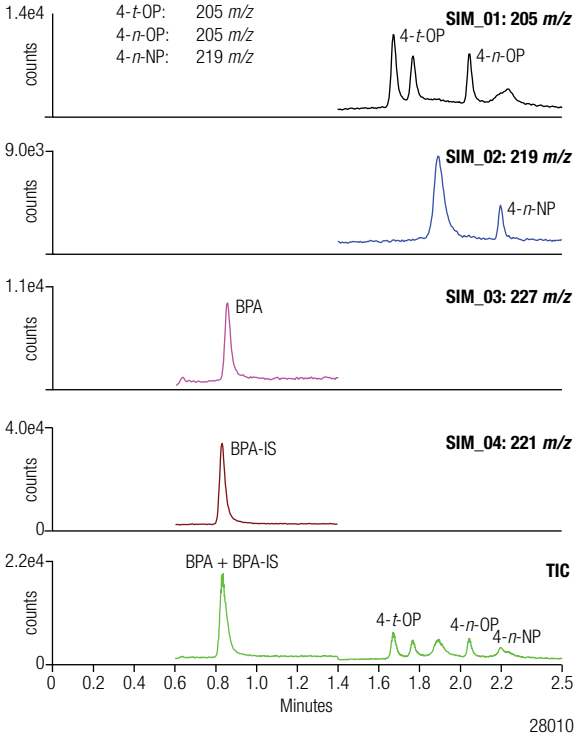


Figure 4-11. SIM and TIC chromatograms of a bottled water sample spiked with 1 ppb BPA and 2 ppb phenols

5. Industrial Applications: Food and Beverage

Acrylamide in Food

Introduction

Acrylamide is a genotoxic compound found in fried or baked goods. It is produced when asparagine reacts with reducing sugars such as fructose or glucose, or carbonyl compounds. Browning the ingredients while cooking produces acylamide, as does overcooking. The acrylamide content in some samples, such as hash browns or french fries, can be particularly high, as much as several mg/kg.

Methods for acrylamide determination have been published by the U.S. EPA (Method 8032A) using liquid extraction, and by the German Health Agency (BGVV), using HPLC with UV detection. The method presented here demonstrates fast, automated extraction procedure using an Thermo Scientific™ Dionex™ ASE™ Accelerated Solvent Extractor.[1] The extracts are analyzed by ICE-MS.

The benefits of this method are simplicity and a high degree of automation, which allows analysis of large numbers of samples with minimal labor.

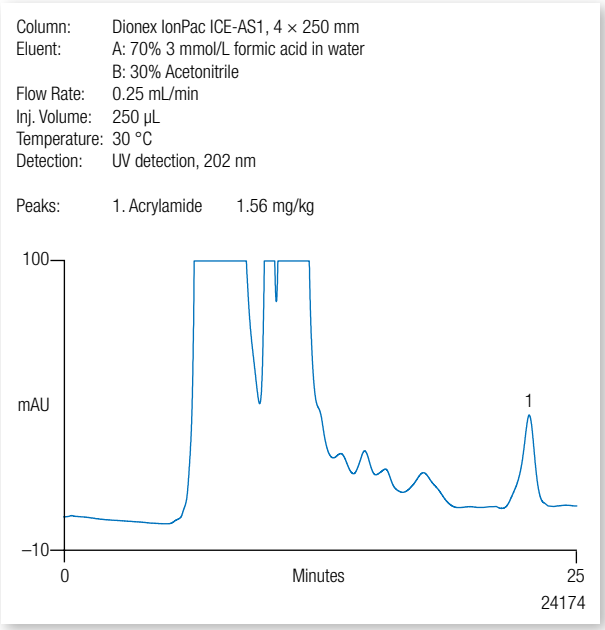


Figure 5-1. French fries sample with high acrylamide content

Analysis

Food samples were extracted using a Dionex ASE Accelerated Solvent Extractor. Chromatographic analysis was performed on a IC system interfaced with an MSQ mass spectrometry detector.

A 4 mm Dionex IonPac ICE-AS1 column was used for separation of acrylamide from the matrix compounds. All measurements were made at 30 °C using formic acid/acetonitrile/water eluent. Figure 5-1 shows a UV chromatogram of a sample of french fries.

The MS ionization mode was ESI positive with a cone voltage of 50 V. Figure 5-2 shows a mass spectrum of acrylamide, where the protonated molecular ion $[M+H]^+$ is detected at 72 *m/z*. The MS can detect very low amounts of acrylamide as shown in Figure 5-3, in a crisp bread sample where the acrylamide content was 0.08 mg/kg.

References

1. Richter, B.E.; Jones, B.A.; Ezzell, J.L.; Porter, N.L.; Avdalovic, N.; Pohl, C. *Anal. Chem.* **1995**, 68, 1033.

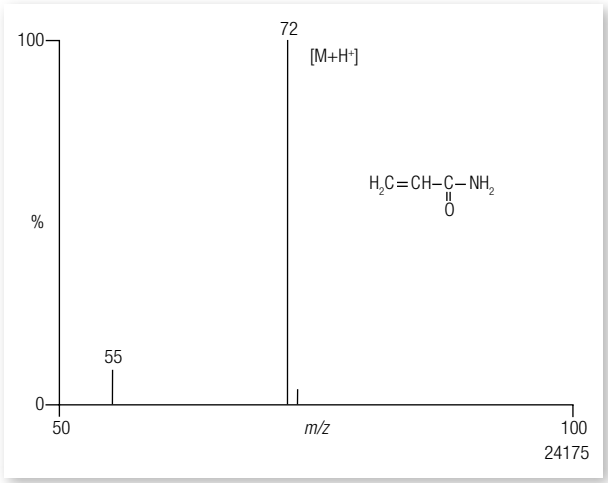


Figure 5-2. Mass spectrum of acrylamide in the range 50–100 *m/z*, ESI positive, cone voltage: 50 V

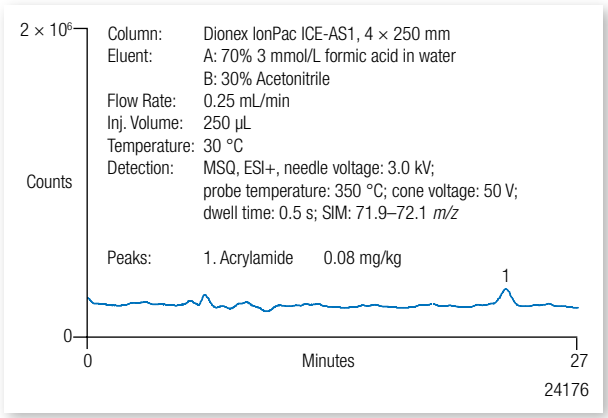


Figure 5-3. SIM chromatogram of crisp bread sample with low acrylamide content

Biogenic Amines in Meat Products

Introduction

Biogenic amines (cadaverine, putrescine, spermidine, histamine, phenyethylamine, agmatine, and tyramine) are organic bases commonly present in living organisms, where they are responsible for many essential functions.

Biogenic amines (BAs) in food and meat products are related to both food spoilage and food safety. Determination of these compounds in fresh and processed food is of great interest, not only because of their toxicity, but also because of their use as spoilage indicators.

Various HPLC methods have been developed for analysis of BAs in foods, but due to lack of suitable chromophoric or fluorophoric groups, almost all of the proposed methods require pre- or postcolumn derivatization to provide sufficient sensitivity.

These examples describe the use of a novel cation-exchange selectivity column coupled with conductivity and MS detection in series.

Analysis

Figure 5-4 shows cation-exchange chromatographic separation of biogenic amines standard solution using gradient elution with suppressed conductivity and MS detection. All amines of interest elute within approximately 25 min. Total run time was extended to 40 min to allow elution of other compounds present in real samples.[1,2]

The LOD ranges from 25–75 µg/L depending on the compound.

Amounts of biogenic amines in raw or cooked ham are generally much lower than those in fermented sausage and far from any toxic level. In Figure 5-5 (conductivity detector), a small amount of agmatine (Peak 4) is observed in cooked ham and confirmed by MS. The spectrum of agmatine is shown in Figure 5-6.

Columns: Dionex IonPac CG17, 2 × 50 mm
Dionex IonPac CS17, 2 × 250 mm
Eluent: MSA from Dionex EG50
Gradient: 7 min isocratic at 3 mmol/L MSA prior to injection, then 6 min isocratic at 3 mmol/L, then in 20 min to 30 mmol/L for additional 14 min
Flow Rate: 0.38 mL/min
Inj. Volume: 5 µL
Temperature: 40 °C
Suppressor: Thermo Scientific™ Dionex™ CSRS™ ULTRA II Cation Self-Regenerating Suppressor, 2 mm, current: 50 mA
Detection: 1) Conductivity
2) MSQ, ESI+, needle voltage: 3.0 kV; probe temperature: 400 °C; cone voltage: 50 V; dwell time: 0.2 s

Peaks:

		m/z
1.	Putrescine	89
2.	Cadaverine	103
3.	Histamine	112
4.	Agmatine	131
5.	Phenethylamine	122
6.	Spermidine	146

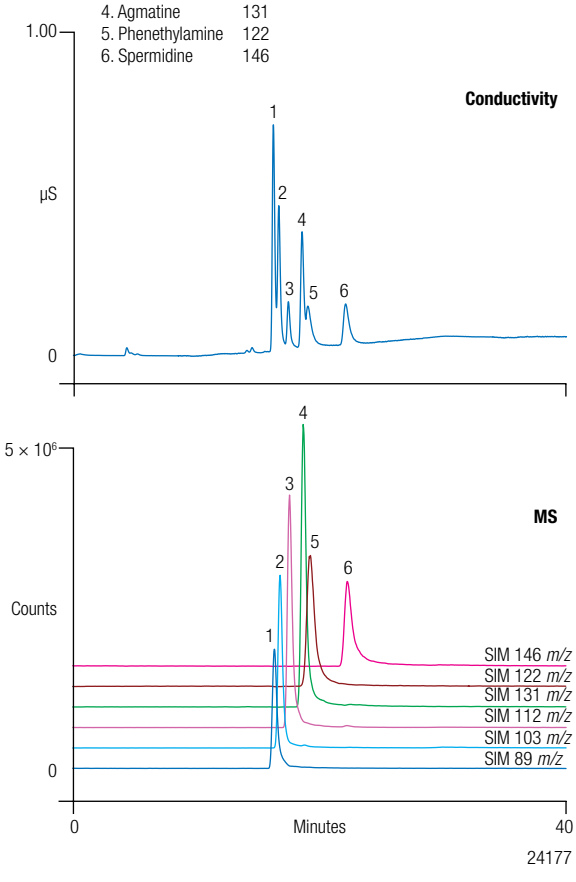


Figure 5-4. Chromatograms of a standard solution (5 mg/L each) of biogenic amines

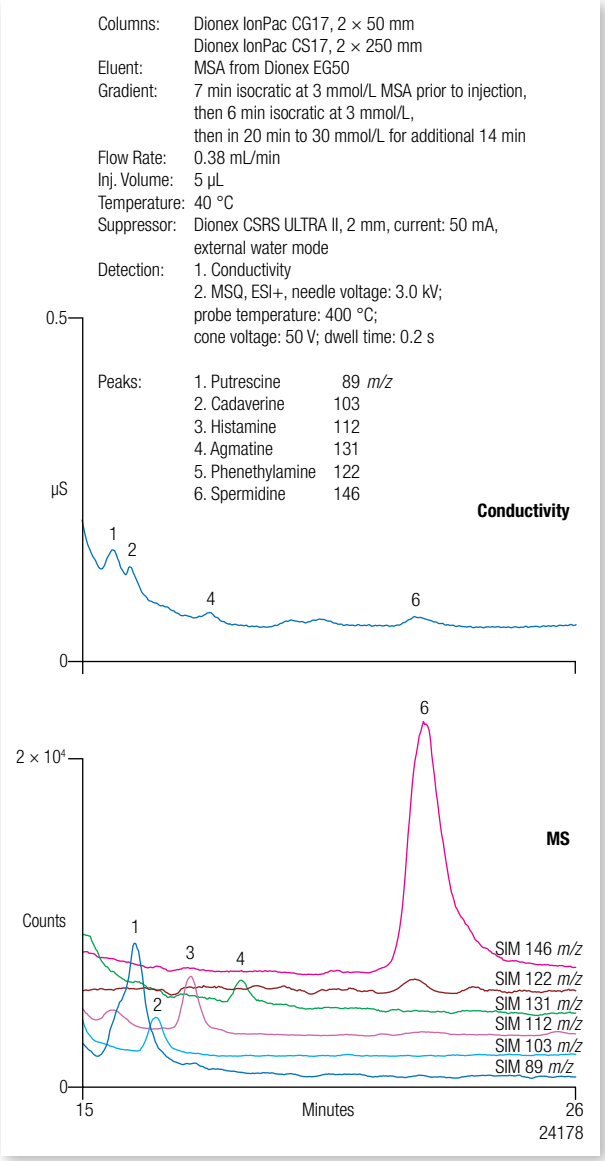


Figure 5-5. Chromatogram of a meat sample

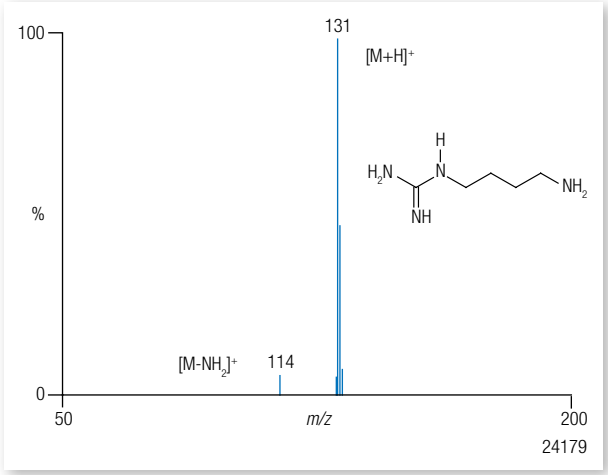


Figure 5-6. Mass spectrum of agmatine, ESI positive, cone voltage: 50 V

References

1. Saccani, G.; Tanzi, E.; Pastore, P.; Cavalli, S.; Compiano, A. M. *Determination of Biogenic Amine in Fresh and Processed Meat by IC-MS Using a Novel Cation-Exchange Column.* Poster presented at 25th International Symposium on Chromatography, Paris, October 4–8, 2004.

2. G. Saccani, E. Tanzi, P. Pastore, S. Cavalli, M. Rey. Determination of Biogenic Amines in Fresh and Processed Meat by Suppressed Ion Chromatography-Mass Spectrometry Using a Cation-Exchange Column. *J. Chromatogr., A.* **2005**, *1082,* 43–50.

Quinine in Tonic Water

Introduction

Quinine, an alkaloid extracted from cinchona bark, was used as one of the very first anti-malaria drugs and is still used today. Quinine can be analyzed by HPLC with UV or fluorescence detection with high sensitivity. For peak confirmation, MS is used.

Analysis

Quinine can be detected in ESI positive mode as the quasi-molecular ion $[M+H]^+$ at 325 *m/z*. At a cone voltage of 75 V, characteristic fragment ions are also detected.

Quinine can be separated on an Acclaim C18 column using 20% methanol, 10% acetonitrile and 70% water. Figure 5-7 shows an overlay of the chromatograms obtained using UV and MS detection in series. The difference in retention times due to the slight delay caused by the flow path between detectors has been corrected using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software. The UV spectrum and the ESI mass spectrum in positive mode are shown in Figures 5-8 and 5-9.

Column:	Acclaim 120, C18, 3 µ, 120 Å, 2.1 × 150 mm
Eluent:	20% Methanol, 10% Acetonitrile, 70% Water
Flow Rate:	0.25 mL/min
Inj. Volume:	10 µL
Temperature:	35 °C
Detection:	1. UV: 254 nm; 3-D: 200–800 nm 2. MSQ, ESI+, needle voltage: 3.0 kV; probe temperature: 350 °C; cone voltage: 50 V; dwell time: 0.2 s; SIM: 324.7–325.7 *m/z*
Peaks:	1. Quinine 325 *m/z*

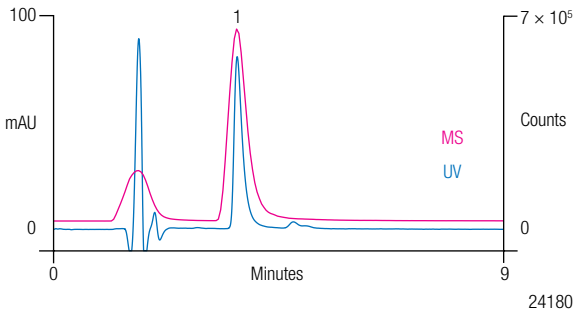


Figure 5-7. Comparison between UV and SIM chromatograms for quinine determination in tonic water

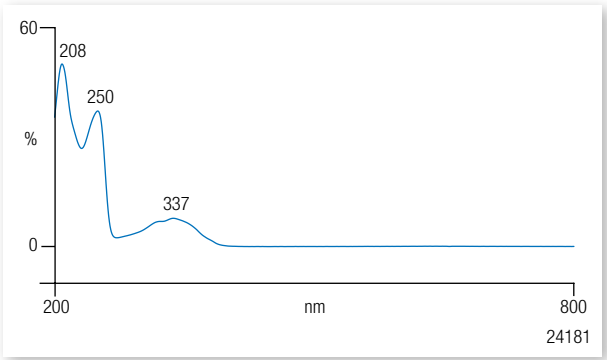


Figure 5-8. UV spectrum of quinine

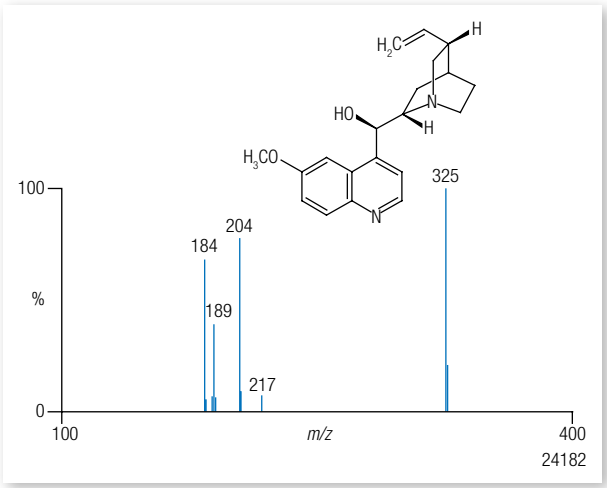


Figure 5-9. MS spectrum of quinine, ESI positive, cone voltage: 75 V, scan time: 1 s, mass range: 100–800 *m/z*

Carbohydrates in Food

Introduction

Carbohydrates are a complex class of organic molecules with the formula $C_nH_{2n}O_n$. They include monosaccharides (trioses, tetroses, pentoses, and hexoses), disaccharides, and oligosaccharides. Carbohydrates are important macronutrients and can be found in most kinds of foods. In addition, sugar alcohols are used as sweeteners and are found in low-calorie diet foods.

Analysis

Carbohydrates can be separated at high pH values as anions on anion-exchange columns using sodium hydroxide and sodium acetate eluents.

High-value pH eluents are not directly compatible for introduction into an MS. Before MS detection, the eluent is first run through a high-capacity supressor (desalter), which lowers the pH to neutral levels.

MS detection is challenging because carbohydrates at neutral pH cannot be directly ionized by ESI. Therefore, 0.5 mmol/L LiCl is added to the desalted eluent postsuppressor to create an ionic species suitable for MS detection.

A typical separation of sugar alcohols, mono- and disaccharides is presented in Figure 5-10.

The carbohydrates are detected as the lithium adducts $[M+7]^+$ in ESI positive mode. In-source fragmentation or CID can also be used to form characteristic fragment ions shown in Figure 5-11, the mass spectrum of maltose.

Columns:	Thermo Scientific™ Dionex™ CarboPac™ PA200, 3 mm × 250 mm, with guard column
Eluent:	A: Water B: 600 mmol/L NaOH C: 500 mmol/L NaOAc
Flow Rate:	0.50 mL/min
Inj. Volume:	20 µL
Temperature:	35 °C
Suppressor:	Dionex ASRS ULTRA II, 2 mm, current: 286 mA, external water mode
Detection:	IPAD, MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 500 °C; cone voltage: 75 V; scan time: 1 s; mass range: 100–200 *m/z*

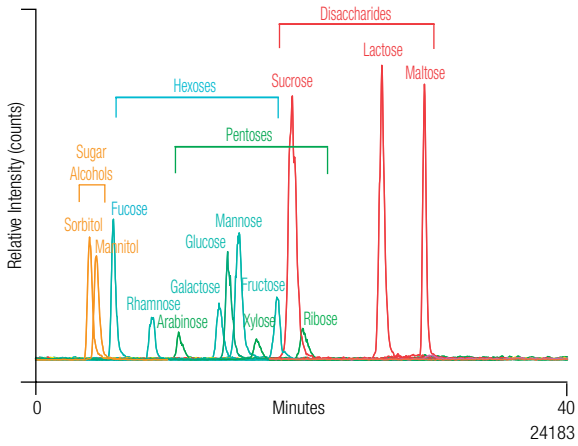


Figure 5-10. EIC of sugar alcohols, mono- and disaccharides in the presence of LiCl, ESI positive, cone voltage: 70 V

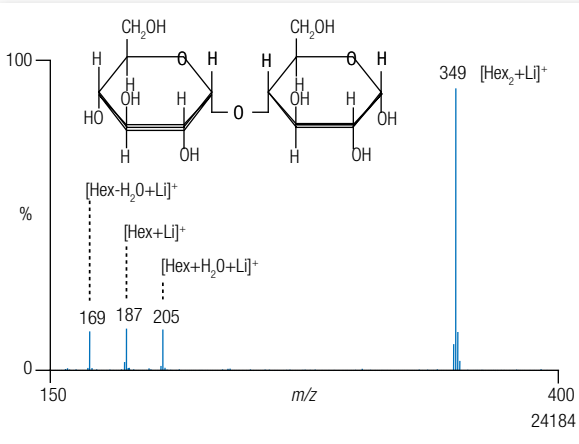


Figure 5-11. Mass spectrum of maltose in the presence of LiCl, ESI positive, cone voltage: 70 V

The separation of inulin carbohydrates (for example, oligosaccharides from chicory) is presented in Figure 5-12, which compares pulsed amperometric and extracted ion current (EIC) profiles from full scan MS detection.

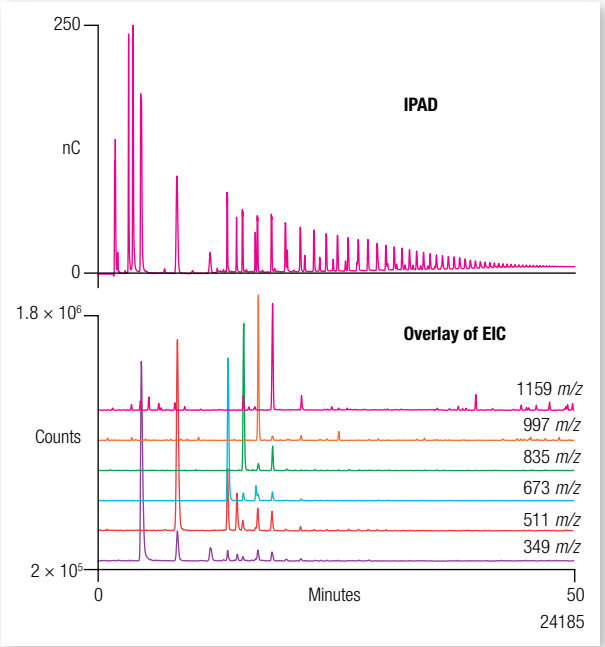


Figure 5-12. Comparison between IPAD and MS detection of chicory inulin, ESI positive, cone voltage: 70 V, scan time: 1 s, mass range: 50–450 *m/z*

Beer contains a large variety of different sugars, including oligosaccharides with up to 10 degrees of polymerization. Figure 5-13 shows an overlay of mass extracted chromatograms of a lager beer sample according to the different degrees of polymerization.

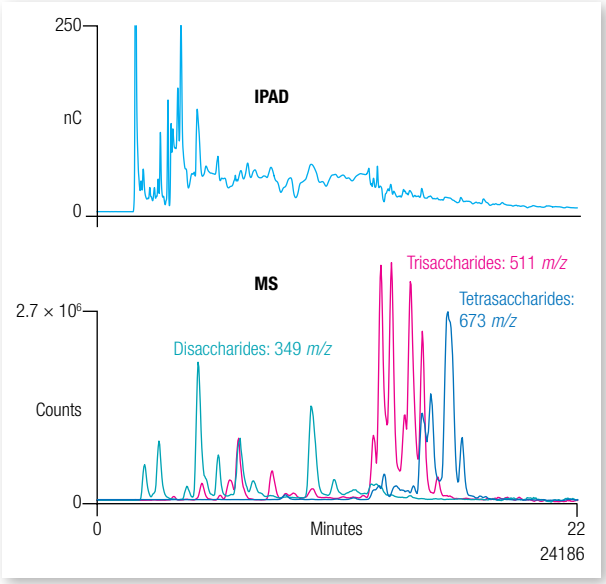


Figure 5-13. Comparison between IPAD and MS detection using degassed lager beer

Amadori Compounds

Introduction

Amadori products are reactive amino acid sugar conjugates formed by the Maillard reaction of an α-hydroxy carbonyl moiety of a reducing sugar and an amino group of an amino acid. This reaction can be initiated even under mild conditions; food products stored at room temperature may eventually turn brown due to the reaction of reducing sugars with amino acids. However, during high-temperature processing of food, further degradation of Amadori products, formed in the early phase of the Maillard reaction, results in the formation of complex mixtures of heterocyclic, polymeric, and other compounds. These products are directly responsible for the distinctive aroma and brown color of baked and roasted food products.

Analysis

Amadori products are polar analytes that are not well separated on typical reversed-phase HPLC columns; therefore, a polar embedded stationary phase (Acclaim PA) was used with 40 mM formic acid in water as mobile phase. Under these conditions, separation of these compounds is still not optimal. However, because they all differ in mass, coelution is not a problem for determining them in a mixture when using MS detection. Figures 5-14 through 5-16 show the mass spectra of three Amadori products obtained with ESI in positive mode with a cone voltage of 50 V. An overlay of the SIM channels for the individual Amadori compounds is shown in Figure 5-17.

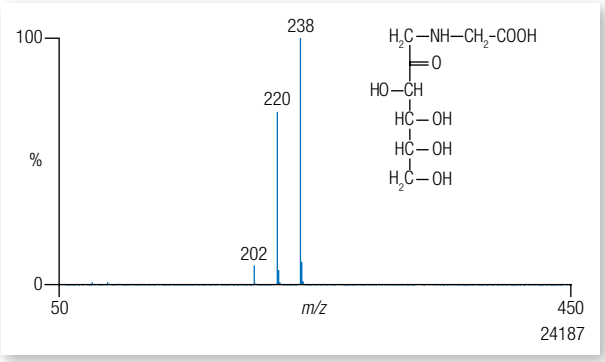


Figure 5-14. Mass spectrum of fru-gly, ESI positive, cone voltage: 50 V, scan time: 1 s, mass range: 50–450 *m/z*

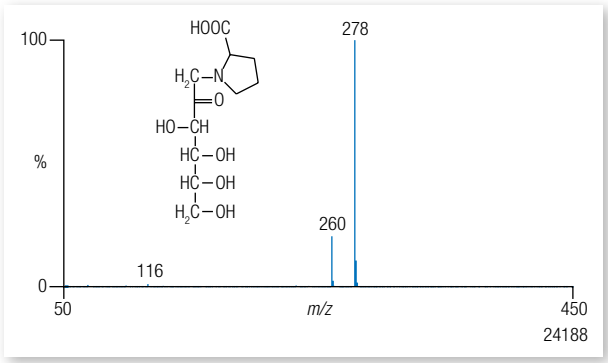


Figure 5-15. Mass spectrum of fru-pro, ESI positive, cone voltage: 50 V, scan time: 1 s, mass range: 50–450 *m/z*

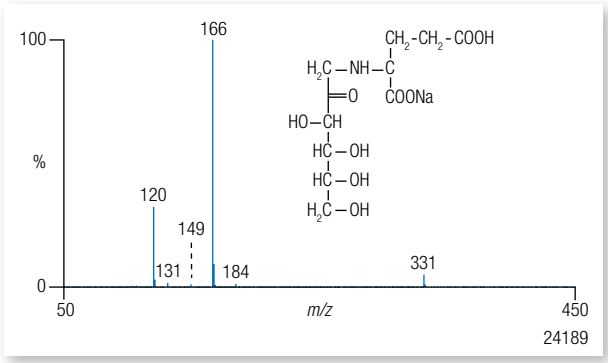


Figure 5-16. Mass spectrum of fru-glu, ESI positive, cone voltage: 50 V, scan time: 1 s, mass range: 50–450 *m/z*

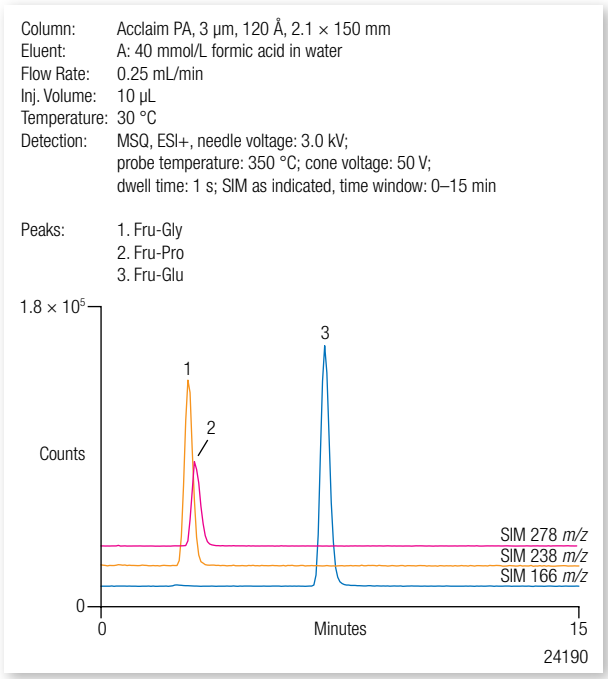


Figure 5-17. SIM chromatogram of different amadori compounds, ESI positive, cone voltage: 50 V

Naturally Occurring Phenolic Acids

Introduction

Phenolic acids serve various roles in plant life, varying from structural to protection. Naturally occurring phenolic acids share the frame structure of hydroxycinnamic acid or hydroxybenzoic acid and are present in many foods and plants. Food quality is a focus of research on phenolic acids since they are associated with color, sensory qualities, nutritional, and antioxidant properties, and potential health implications.

Analysis

This application uses mixed-mode chromatography to retain and resolve 15 phenolic acids with a mild acidic mobile phase (pH 4), which improves MS detection under negative ionization mode to ensure the method's selectivity and sensitivity.

Phenolic acids have the carboxylic functional group, which provides substantial hydrophilicity and causes the difficulty of reversed-phase retention. Because the WAX-1 column features both reversed-phase and weak anion-exchange retention mechanisms, retention and separation can be achieved at a neutral or mild acidic condition, which facilitates MS response under negative ionization mode. The separation and detection parameters are as presented on the chromatogram.

The green tea sample was steeped in 100 mL hot water for 10 min, then vortexed and filtered, diluted 1 to 10 in DI water, and injected directly for analysis.

Results

Figure 5-18 shows the UV and SIM chromatograms of 15 phenolic acids used for this study. Figure 5-19 shows the calibration curve for m-coumaric acid. Seven replicate injections of a standard mixture were performed to calculate MDL for each analyte. Method detection limit was calculated by the equation:

$$MDL = t_{99\%} \times S_{(n=7)}$$

where t is Student's t at 99% confidence intervals ($t_{99\%,\ n=7} = 3.143$) and S is the standard deviation. Detection limits were calculated at 0.2 ~ 1.0 ng for all listed organic acids except for 4-hydroxyphenyllactic acid which has a detection limit at 2.25 ng. This method was evaluated for quantitative analysis of phenolic acids found in beverages. Figure 5-20 shows the chromatogram for green tea analysis.

Analytical Column: Acclaim Mixed-Mode WAX-1, 150 × 2.1 mm
Eluent: 83% CH_3CN, 5% 200 mM NH_4OAc pH 4.6, 12% H_2O
Flow Rate: 0.5 mL/min
Column Temperature: 50 °C

Peak	R.T.	SIM	Peak	R.T.	SIM
1. Sinapic acid	5.7	223.1	9. Benzoic acid	10.3	121.0
2. Ferulic acid	6.2	193.1	10. *o*-coumaric acid	10.9	163.1
3. Veratric acid	6.7	181.1	11. *m*-coumaric acid	11.6	163.1
4. *p*-coumaric acid	7.3	163.1	12. Caffeic acid	11.8	179.0
5. Vanillic acid	7.5	167.0	13. Salicylic acid	12.5	137.1
6. Syringic acid	7.5	197.1	14. Protocatechuic acid	14.0	153.0
7. *p*-hydroxybenzoic acid	8.1	137.1	15. Gentisic acid	16.3	153.0
8. Cinnamic acid	8.8	147.1			

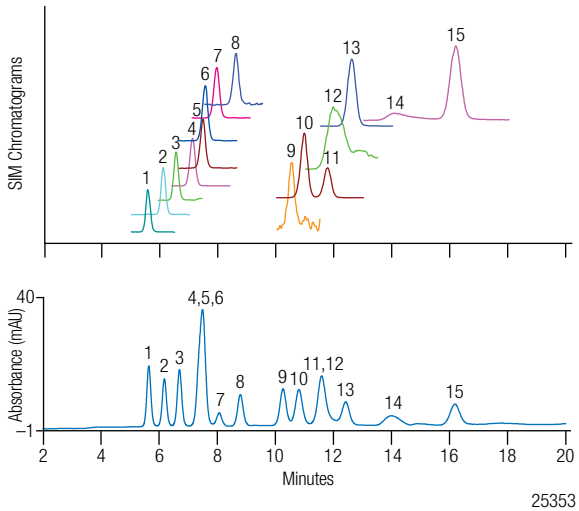


Figure 5-18. SIM and UV chromatograms of 15 predominant naturally occurring phenolic acids. 5 ng injected for MS SIM detections, 25 ng injected for UV detection. SIM chromatogram is normalized to 100% of the greatest peak in each channel.

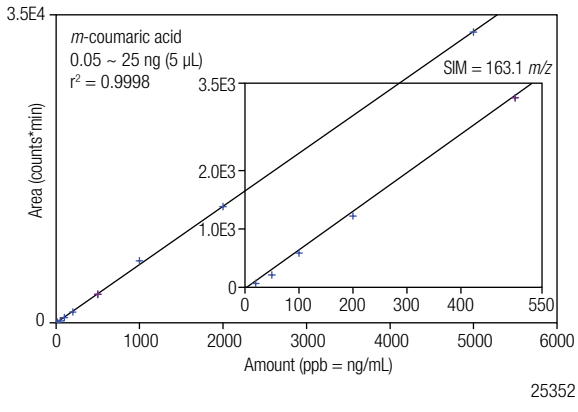


Figure 5-19. Calibration curve for *m*-coumaric acid

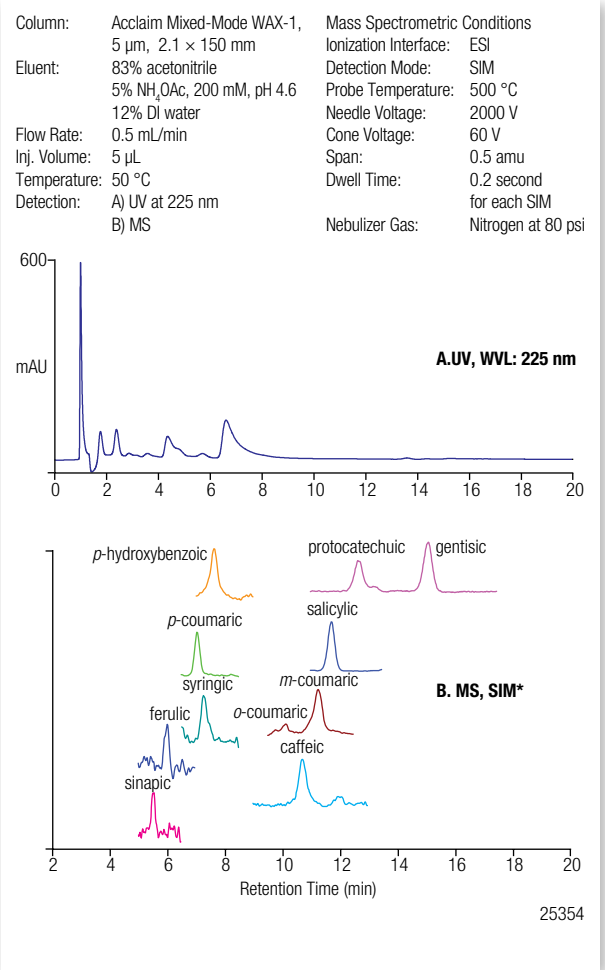


Figure 5-20. Chromatogram for green tea analysis

Melamine and Cyanuric Acid

Introduction

Recent investigations of pet animal deaths and health problems have revealed pet food contaminated by melamine and cyanuric acid. When present together, melamine and cyanuric acid form an insoluble crystal, which may cause kidney failure. Since contaminated wheat gluten, rice protein concentrate, and corn gluten used in animal feed can be also used in human food (for example, bread, pasta, and baby food), it is crucial to monitor the presence of melamine and cyanuric acid in raw materials and animal tissue.

Analysis

The pet food samples were ground and then extracted using a Dionex ASE 300 Accelerated Solvent Extractor with acetonitrile/water = 80/20 at a temperature of 110 °C and pressure of 1500 psi for 5 min. They were then flushed with 50% cell volume and purged with nitrogen for 140 s. The total extract from two cycles of the above procedure was collected and brought up to a final volume of 40 mL. The sample extracts were filtered through a 45 µm nylon filter and were then ready for analysis.

Melamine and cyanuric acid are well separated on an Acclaim Mixed-Mode WAX-1 column in the HILIC mode (90% acetonitrile v/v). Due to the novel column chemistry, chromatographic separation can be optimized by adjusting the mobile phase ionic strength, pH, or organic content. The reported optimal chromatographic condition was the result of a combination of good resolution, speed of analysis, good MS response, and minimal ion suppression from matrix effects. Parameters are listed in Figure 5-22.

Results

Structures of melamine and cyanuric acid are shown in Figure 5-21, along with the full scan MS spectra.

Figure 5-22 shows the SIM chromatogram of an FDA control sample spiked with melamine, cyanuric acid, and internal standards.

Figures 5-23 and 5-24 show the calibration curves for melamine and cyanuric acid. Melamine and cyanuric acid were eluted with little matrix interference at 3.86 and 6.18 min, with capacity factors of 2.0 and 6.1, respectively. Under the current conditions, the detection limits were shown to be approximately 1 ppb for melamine and 15 ppb for cyanuric acid.

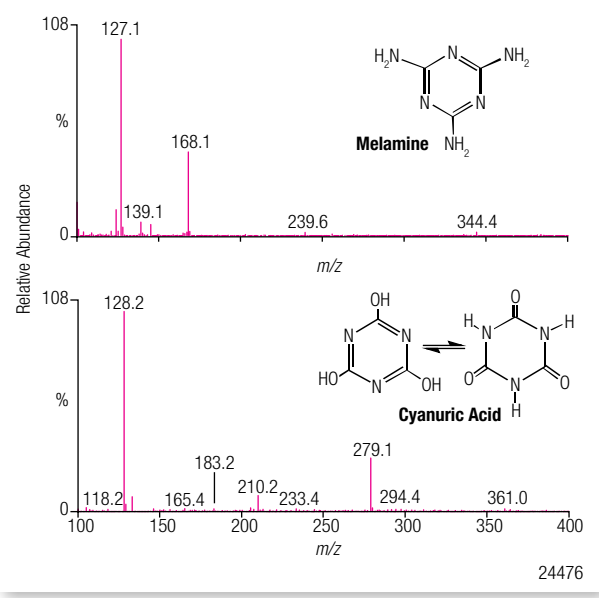


Figure 5-21. Structures of melamine and cyanuric acid and full scan MS spectra

Column: Acclaim Mixed-Mode WAX-1, 5 µm, 2.1 × 150 mm
Eluent: 90% Acetonitrile
10% Ammonium acetate buffer, 20 mM, pH 4
Flow Rate: 0.25 mL/min
Inj. Volume: 5 µL
Temperature: 20 °C
Detection: MS

Mass Spectrometric Conditions
Ionization Interface: ESI
Detection Mode: SIM
Probe Temperature: 500 °C
Needle Voltage: 2000 V
Cone Voltage: 50 V
Dwell Time: 0.5 s for each SIM

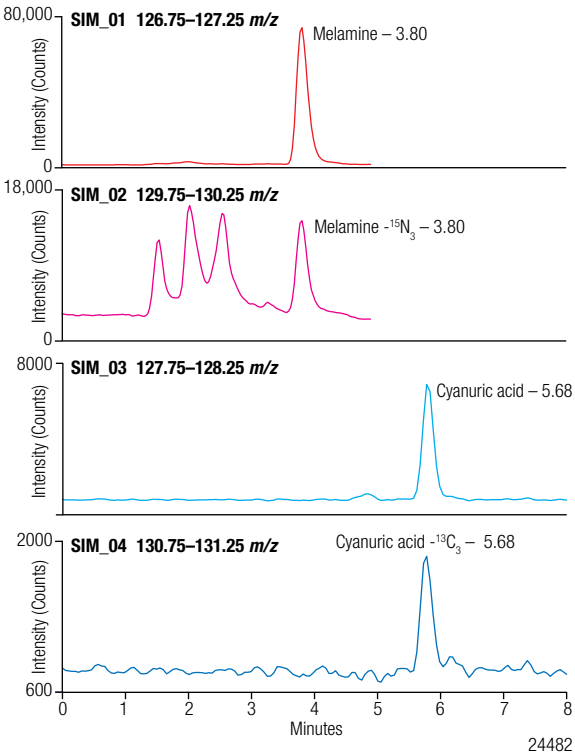


Figure 5-22. Structures of melamine and cyanuric acid and full scan MS spectra

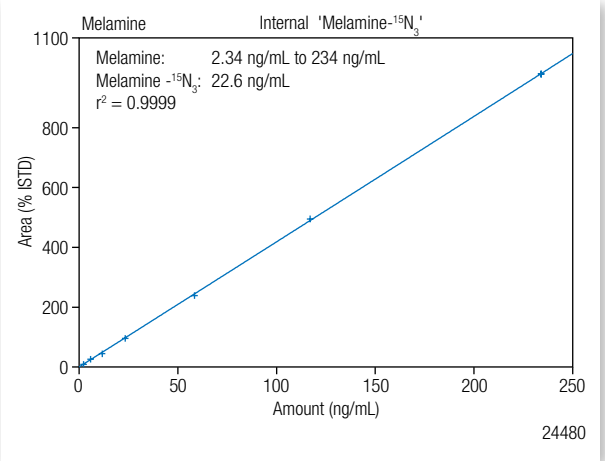


Figure 5-23. Calibration curve for melamine

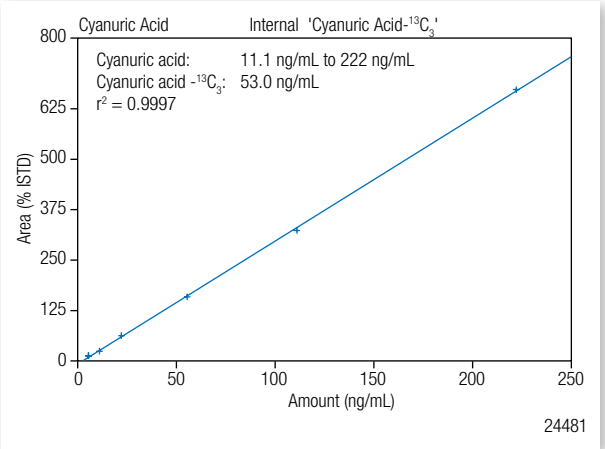


Figure 5-24. Calibration curve for cyanuric acid

Perchlorate in Baby Formula and Milk

Introduction

Perchlorate has been found in various media and is now considered a widespread contaminant in the United States. Perchlorate poses an adverse effect on human health by competitively inhibiting iodide uptake and reducing thyroid hormone production, which is essential for proper protein expression, neuronal differentiation, and other functions. Certain populations, such as infants and children, may be at higher risk due to greater dependency on milk products and higher consumption/body weight ratio.

This application describes an IC-MS/MS method for ultratrace-level perchlorate analysis in both liquid and powdered baby formula.

Analysis

The powdered infant formula (PIF) sample was prepared to liquid form with DI water according to manufacture-provided instructions. Each sample (4 mL) was pipetted into a 50 mL centrifuge tube and spiked with 40 µL of IStd (4 ng). For protein precipitation, 4 mL of precooled ethanol (stored under refrigeration at 4 °C) and 0.4 mL of 3% acetic acid (diluted in DI water to 3% for sample preparation) were added to each sample. Each sample was then mixed in a vortex mixer and centrifuged at 5000 rpm for 30 min at –5 °C. The supernatant was passed through a 0.25 µm preconditioned syringe filter and a Thermo Scientific™ Dionex™ OnGuard™ RP II cartridge, collected in a 10 mL autosampler vial, and analyzed by IC-MS/MS.

Results

Calibration standards were prepared at 0 ppt, 20 ppt, 50 ppt, 100 ppt, 200 ppt, 500 ppt, 1 ppb, 2 ppb, 5 ppb, and 10 ppb with all standards containing IStd at 1 ppb at each level.

Figure 5-25 shows the SRM chromatograms of an infant formula and a milk sample. Isotope-labeled internal standard was used for quantification and spiked in each sample at 1 ppb. Perchlorate was quantified at 2.44 ppb in the infant formula sample and 4.65 ppb in the milk sample.

Figure 5-26 demonstrates the calibration curve from 20 ppt to 10 ppb with excellent correlation coefficient at $r^2 = 0.9996$ and 0.9998 for two SRM channels.

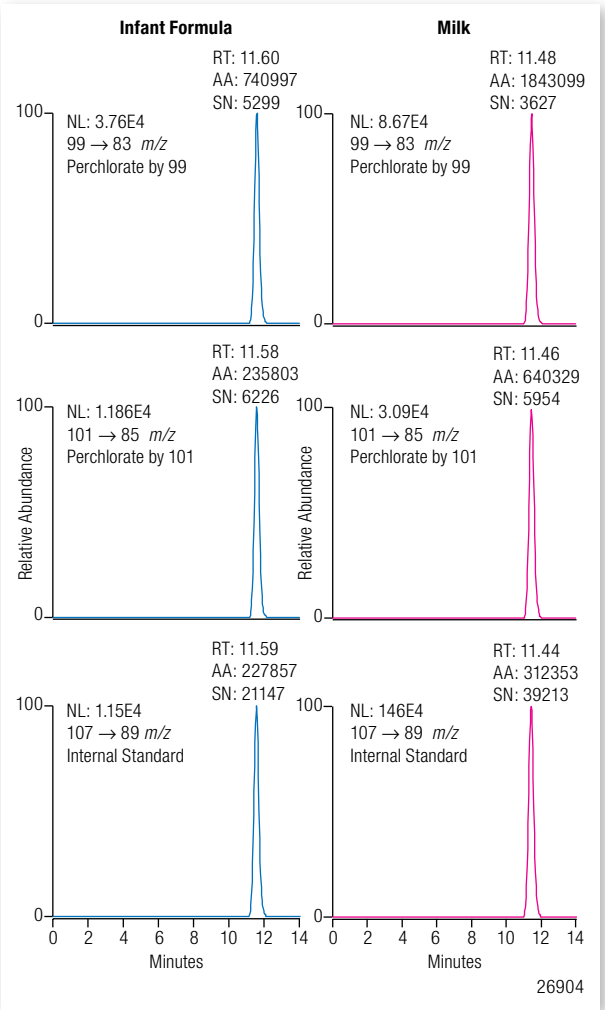


Figure 5-25. Perchlorate in infant formula and milk. Perchlorate was quantified at 2.44 ppb in this infant formula sample and 4.65 ppb in this milk sample.

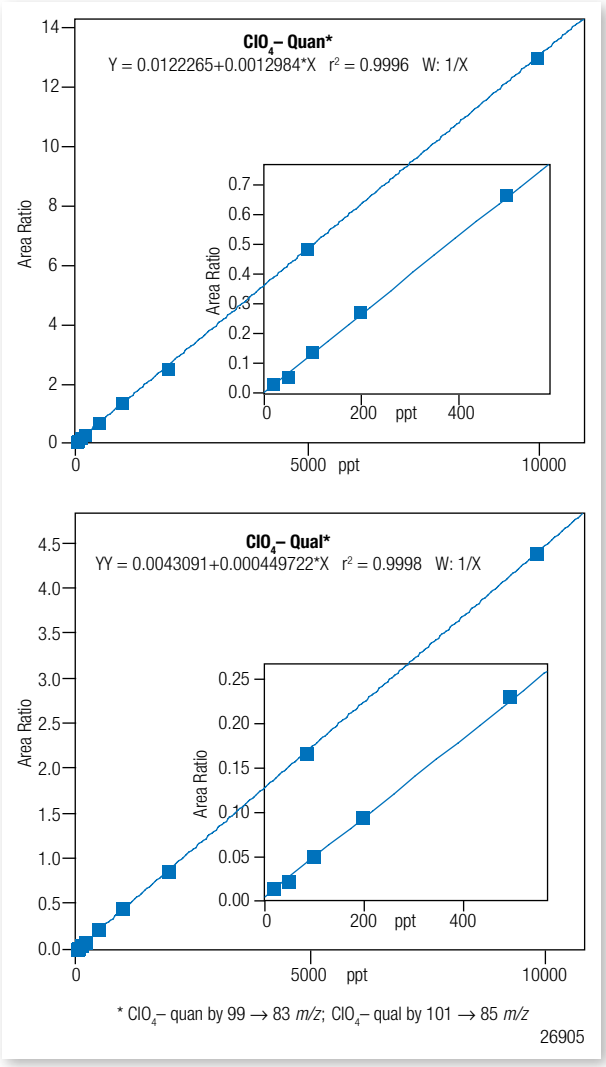


Figure 5-26. Perchlorate in infant formula and milk. Perchlorate was quantified at 2.44 ppb in this infant formula sample and 4.65 ppb in this milk sample.

6. Industrial Applications: Chemicals

Amines in Ni/Zn Galvanic Baths

Introduction

Acrylamide is a genotoxic compound found in fried or baked goods. Zinc coating is used extensively to protect milled steel from atmospheric corrosion. Conventional cyanide plating was popular until the 1970s, when a strong international drive to achieve better pollution control led to the development of noncyanide plating baths. Attempts have been made to develop acid sulfate, chloride, and alkaline noncyanide zinc plating baths with varying degrees of success.

Noncyanide alkaline baths require high concentrations of brighteners, and the bright current density range (the total number of amperes required in the bath for optimum plating efficiency) is very narrow. The enclosed data show results pertaining to the use of 2-mercaptobenzothiozole–thiourea, sodium diethyl dithiocarbamate–triethanolamine, and p-methoxysalicylaldehyde–diethanolamine as brighteners for alkaline noncyanide zinc plating bath.

Figure 6-1 shows a suppressed conductivity chromatogram of a galvanic bath with high concentrations of nickel and zinc. This approach does not provide sufficient sensitivity for this type of sample, while MS detection (Figure 6-5) shows all amines present, along with sodium adduct, which elutes near the void volume.

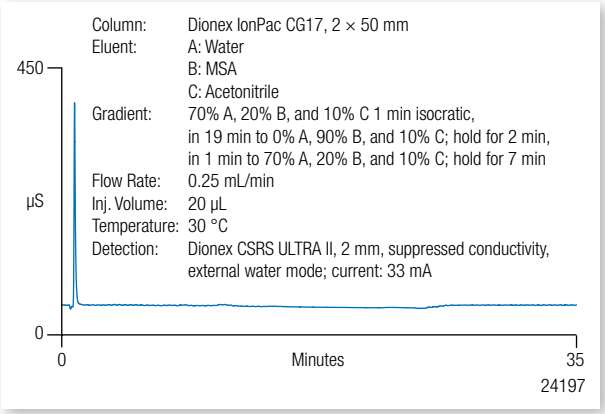


Figure 6-1. Suppressed conductivity chromatogram of different amines in a galvanic bath with concentrated Ni and Zn

Analysis

Analysis was performed on a Thermo Scientific Dionex IC system coupled with the MSQ mass spectrometry detector. Separation was achieved using a 2 mm Dionex IonPac CS17 column with gradient elution of methanesulfonic/acetonitrile eluent. Detection was achieved using both suppressed conductivity and ESI positive MS detection with cone voltage set at 50 V. MS spectra of amines show a prevalence of sodium adduct $[M+Na]^+$ signal with respect to the quasi-molecular ion $[M+H]^+$ (Figures 6-2 through 6-4).

Figure 6-5 shows the same sample as Figure 6-1, with MS SIM chromatograms of triethanolamine, tetraethylene pentamine, and pentaethylene hexamine in a Ni/Zn plating bath.

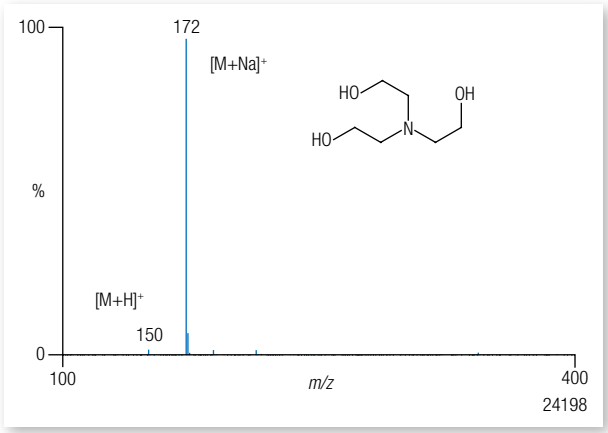


Figure 6-2. Mass spectrum of triethanolamine ESI positive, cone voltage: 50 V

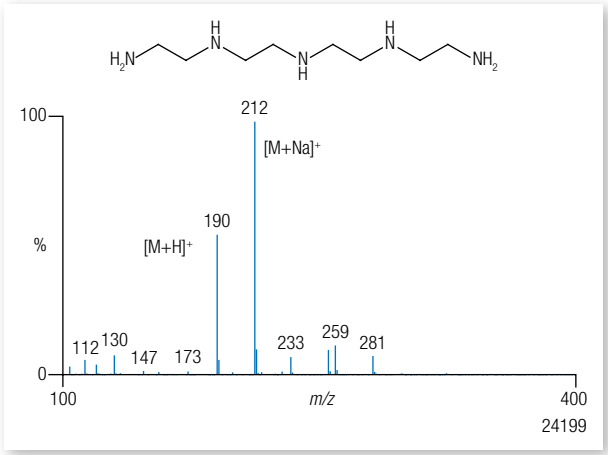


Figure 6-3. Mass spectrum of tetraethylene pentamine, ESI positive, cone voltage: 50 V

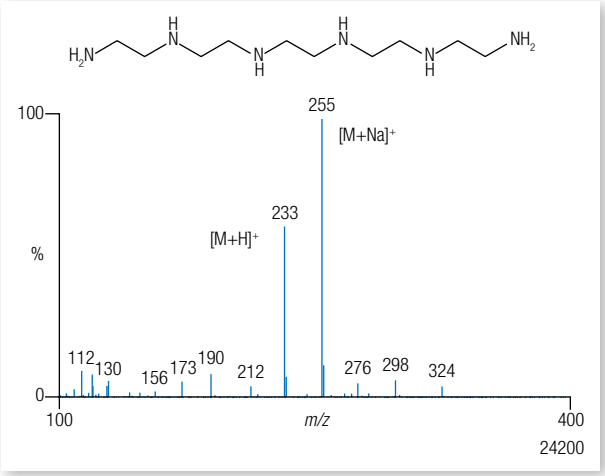


Figure 6-4. Mass spectrum of pentaethylene hexamine, ESI positive, cone voltage: 50 V

Columns:	Dionex IonPac CG17, 2 × 50 mm
Eluent:	A: Water B: MSA C: Acetonitrile
Gradient:	70% A, 20% B, and 10% C 1 min isocratic, in 19 min to 0% A, 90% B, and 10% C; hold for 2 min, in 1 min to 70% A, 20% B, and 10% C; hold for 7 min
Flow Rate:	0.25 mL/min
Inj. Volume:	20 µL
Temperature:	30 °C
Detection:	MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 400 °C; cone voltage: 50 V; dwell time: 0.5 s

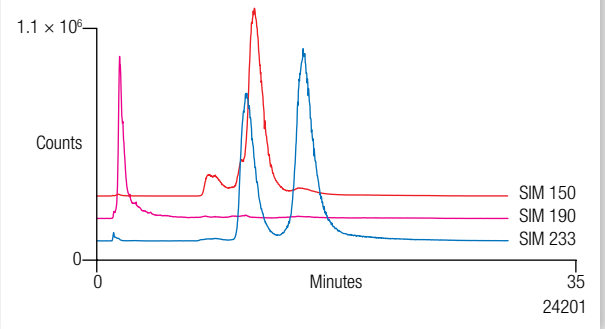


Figure 6-5. SIM chromatograms of different amines in a plating bath consisting of a high concentrated Ni and Zn matrix

Thiosulfate in Concrete

Introduction

Portland cements contain chromium compounds that can release hexavalent chromium (CrVI) in solution over time.[1] Even in very small amounts, soluble chromates can adversely affect human health. Since 1999, the countries of the European Union have labeled packaged cement containing more than 2 mg/kg of soluble CrVI. More recently, the European Parliament and Council have published a new directive (2003/53/EC) introducing strong limitations on the use of cements containing more than two mg/ kg of soluble CrVI. These limitations have been solved with the use of different analytical techniques. The use of IC coupled with MS detection allows determination of chromium and identification of other anionic contaminants derived from raw materials.

Analysis

Figure 6-6 shows a sample of Portland cement analyzed with suppressed conductivity detection. Based on its mass spectrum (Figure 6-7), peak 1 was identified as thiosulfate.

IC-MS determination of thiosulfate and chromate was achieved with the Thermo Scientific Dionex IC system and a Dionex IonPac AS9-HC column with both suppressed conductivity and ESI negative MS detection; cone voltage was set at 50 V. Results were confirmed in a SIM chromatogram (Figure 6-8) obtained by monitoring both quasi-molecular ion mass-to-charge ratios of thiosulfate and chromate.

A confirmation run using MS SIM chromatograms monitoring both quasimolecular ions of thiosulfate and chromate ions is shown in Figure 6-8.

References

1. Lund, M. H. Chromium in Cement. *Nordisk Belong.* **1977**, *6*, 28.

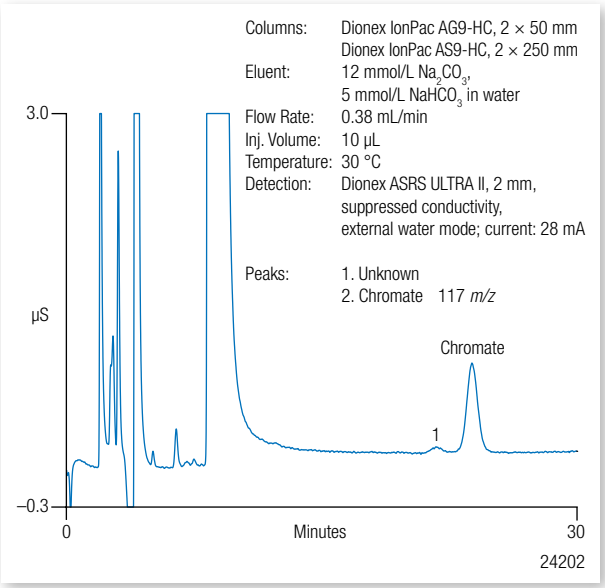


Figure 6-6. Suppressed conductivity chromatogram of cement sample

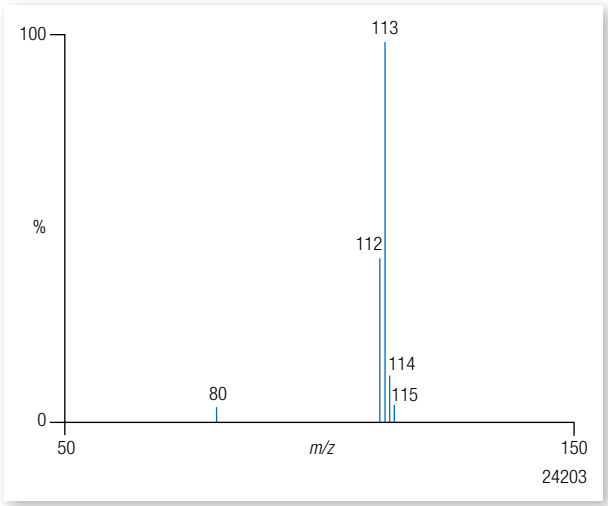


Figure 6-7. Mass spectrum of thiosulfate ESI negative, cone voltage: 50 V

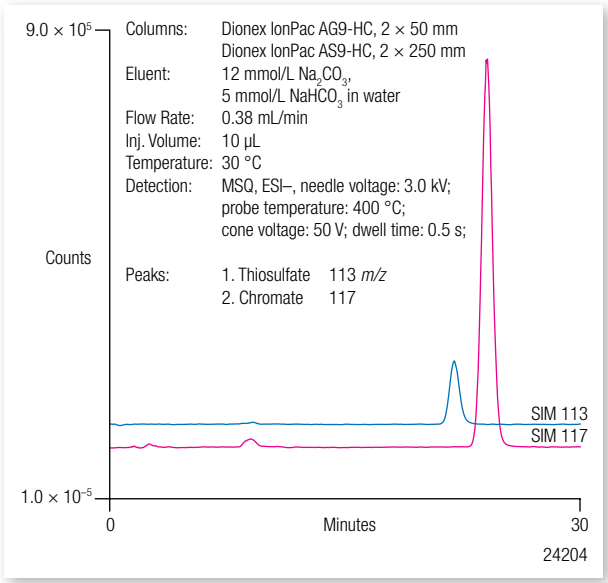


Figure 6-8. Overlay of two chromatograms of Portland cement sample

Polymer Additives

Introduction

Polymer additives are used as processing and long-term thermal stabilizers to protect the polymer from breakdown caused by UV light or oxidation. Tinuvin® is a common UV stabilizer used as an additive (e.g., in polyethylene and polypropylene). Irganox® is the trade name for a class of phenolic-based antioxidants used widely as additives (e.g., in PET and polyolefins). To control formulation levels and to conduct stability studies, the additive content of the polymer must be determined.

Figure 6-9 shows separation of four polymer additives at concentrations of 5 mg/L (Irganox 245, Tinuvin 234, Irganox 259, and Irganox 1010).

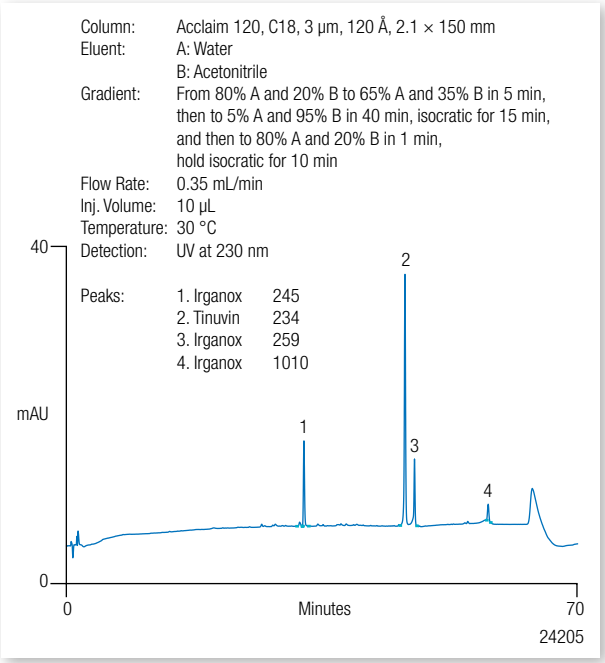


Figure 6-9. UV Chromatogram of different polymer stabilizers

Analysis

Typical sample sizes of 1–5 g polymer are extracted using accelerated solvent extraction according to Dionex (now part of Thermo Scientific) Application Note 331. The additives can be separated by HPLC with the Acclaim C18 column using a water/acetonitrile gradient. For identification and analysis of decomposition products of polymer additives, MS detection is required with APCI in positive mode. The full scan mass spectra of the four polymer additives are shown in Figures 6-10 through 6-13.

Corona current was set at 5 µA with a cone voltage of 35 V.

An overlay of the different SIM channels recorded using the most intense mass is shown in Figure 6-14.

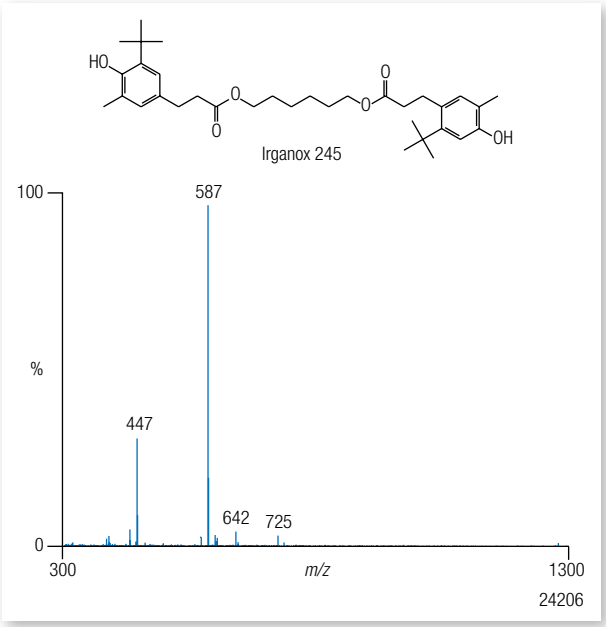


Figure 6-10. Mass spectrum of Irganox 245. Scan time: 0.3 s, cone voltage: 35 V mass range: 300–1300 *m/z*.

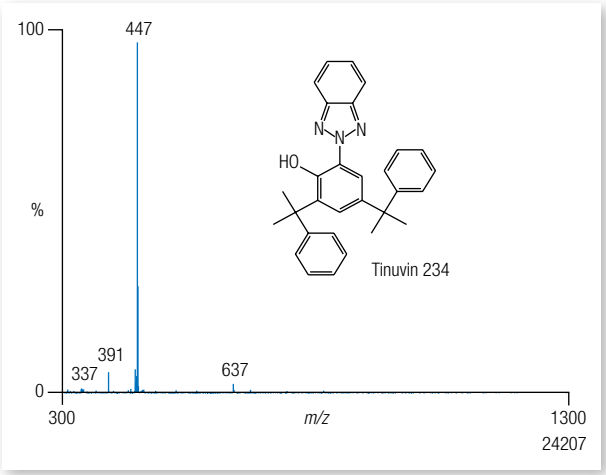


Figure 6-11. Mass spectrum of Tinuvin 234

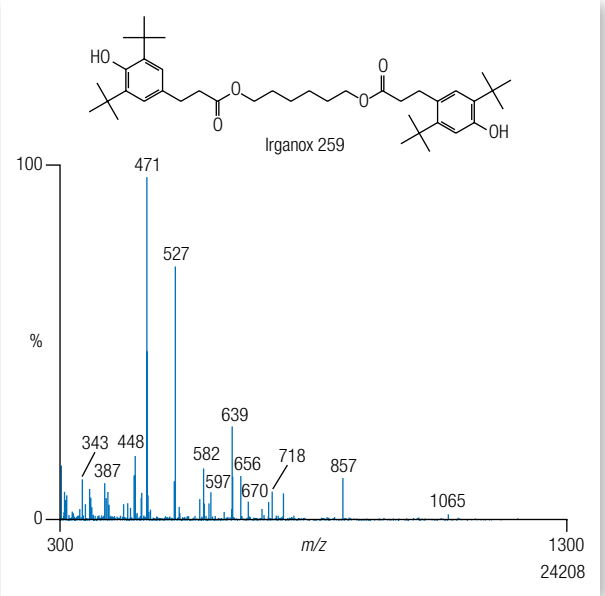


Figure 6-12. Mass spectrum of Irganox 259

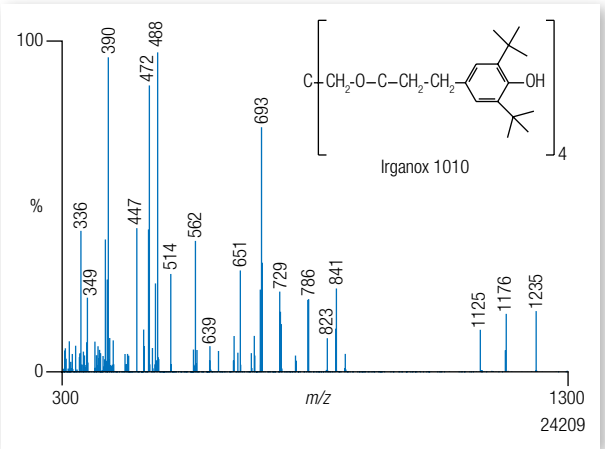


Figure 6-13. Mass spectrum of Irganox 1010

Column:	Acclaim 120, C18, 3 µm, 120 Å, 2.1 × 150 mm
Eluent:	A: Water
	B: Acetonitrile
Gradient:	From 80% A and 20% B to 65% A and 35% B in 5 min, then to 5% A and 95% B in 40 min, isocratic for 15 min, and then to 80% A and 20% B in 1 min, hold isocratic for 10 min
Flow Rate:	0.35 mL/min
Inj. Volume:	10 µL
Temperature:	30 °C
Detection:	MSQ, APCI +, corona current: 5 µA; probe temperature: 450 °C; cone voltage: 35 V; dwell time: 0.3 s; SIM as indicated

Peaks:		
	1. Irganox 1010	488 *m/z*
	2. Irganox 259	471
	3. Irganox 245	587
	4. Tinuvin 234	448

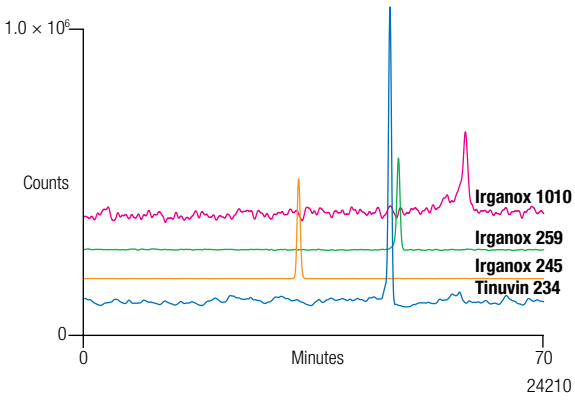


Figure 6-14. SIM chromatogram of different polymer stabilizers

Lauryl Sulfate

Introduction

Lauryl sulfate, in conjunction with the ammonium or sodium counterion, is commonly used as an anionic surfactant. Ammonium lauryl sulfate is used as a foaming agent and a detergent in shampoos, toothpastes, and skin cleansers. Lauryl sulfate is also used to stabilize pesticide formulations and can be found in food products.

Analysis

Lauryl sulfate can be separated on a reversed-phase C18 column isocratically using a mobile phase of 85% acetonitrile and 15% water containing 1 mmol/L ammonium acetate. Under these conditions, nominal retention time is 1.5 min. MS detection can be performed using APCI or ESI in the negative mode with a cone voltage of 50 V. The recorded ESI mass spectrum is presented in Figure 6-15, showing the quasi-molecular ion $[M-H]^-$ at 265 *m/z*.

Figure 6-16 shows an overlay of both ESI and APCI using a lauryl sulfate standard solution of 0.5 mg/L in water. Both ionization techniques are suitable for detection, but using ESI provides a better signal-to-noise ratio.

A linear calibration plot was obtained in the range from 0.2 mg/L to 20 mg/L presented in Figure 6-17.

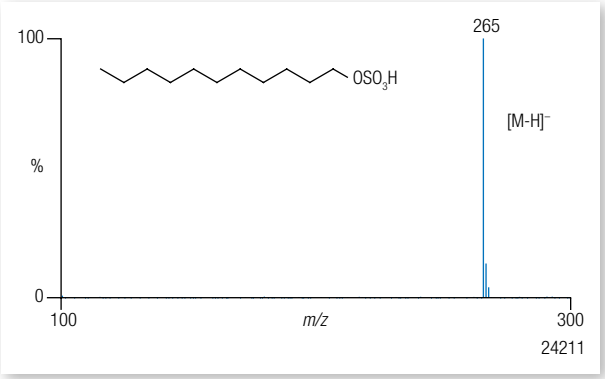


Figure 6-15. Mass spectrum of lauryl sulfate, ESI positive; cone voltage: 50 V scan time: 1 s, mass range: 100–300 *m/z*

Column: Acclaim 120, C18, 3 µm, 120 Å, 2.1 × 100 mm
Eluent: A: 15% 1 mmol/L ammonium acetate in water
B: 85% Acetonitrile
Flow Rate: 0.30 mL/min
Inj. Volume: 10 µL
Temperature: 20 °C
Probe Temp: 400 °C
Detection: MSQ, ESI–, needle voltage: 3.5 kV;
probe temperature: 400 °C;
cone voltage: 50 V;
dwell time: 0.2 s;
SIM 265 *m/z*
APCI current: 10 µA

Peaks: 1. Lauryl sulfate, 0.5 mg/L,
SIM 265.34–265.66 *m/z*

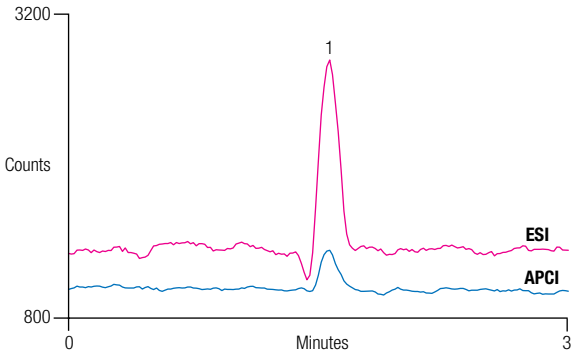


Figure 6-16. Comparison between ESI and APCI sensitivity

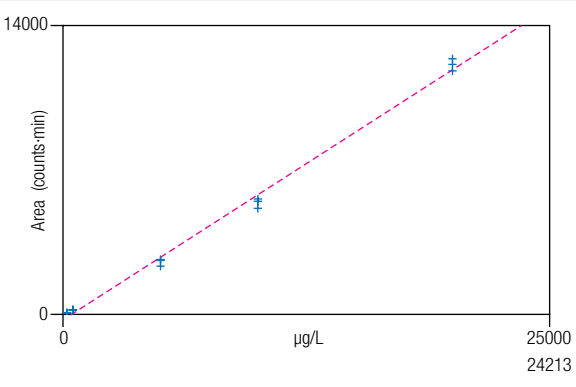


Figure 6-17. Calibration plot of lauryl sulfate.
Range: 0.2–20.0 mg/L, ESI negative, cone voltage: 50 V.

Low Molecular Mass Organic Acids

Introduction

Organic acids are common components of plant and animal tissue. Low molecular mass organic acids (LMMOAs) are present in many plants and serve various functions. They are also found as degradation products during pretreatment processes for biofuel production. Many of these acids inhibit the microbial fermentation process and thus reduce the conversion efficiency to biofuel. To better understand and optimize the conversion process, it is necessary to monitor the LMMOA profile during biofuel production.

Analysis

An ion chromatography method was developed to resolve most analytes, especially analytes with identical or close molecular mass. These analytes cannot be resolved by single quadrupole MS or by MS/MS because they either do not fragment or have no uniquely specific fragmentation pattern. A Dionex ICS-2000 IC system was operated in external water mode, with DI water as a regenerant, at a flow rate of 0.5 mL/min delivered by a Dionex AXP-MS auxiliary pump. A 0.20 mL/min flow of acetonitrile (to assist desolvation) was delivered by a second Dionex AXP-MS auxiliary pump and mixed with the eluent by a static mixing tee prior to entering the mass spectrometer. The mass spectrometer was operated in SIM mode to provide the most sensitive and selective detection.

Columns:	Dionex IonPac AS11-HC and AG11-HC, 2 mm Dionex CR-ATC Continuously Regenerated Anion Trap Column
Mobile Phase:	Hydroxide gradient electrolytically generated from EGC II KOH cartridge

Time (min)	Conc. (mM)
–10	1
8	1
18	15
28	30
38	60
40	1

Flow Rate:	0.38 mL/min
Inj. Volume:	25 µL
Oven Temperature:	30 °C
Detection:	Bottom: Suppressed conductivity Top: MSQ Plus single quadrupole mass spectrometer SIM

Mass Spectrometric Conditions

Solvent:	Acetonitrile at 0.2 mL/min by Dionex AXP-MS pump
Ionization Interface:	ESI
Probe Temperature:	450 °C
Needle Voltage:	3000 V
Scan Mode:	Negative SIM
Nebulizer Gas:	Nitrogen at 80 psi

Analyte	R.T. min	SIM *m/z*	Analyte	R.T. min	SIM *m/z*
quinate	6.9	191.1	glutarate	21.6	131.0
gluconate	7.7	195.1	mucate	21.6	209.0
lactate	8.2	89.0	adipate	21.7	145.1
2-methyllactate	8.2	103.1	succinate	21.9	117.0
acetate	8.8	59.0	malate	22.1	133.0
glycolate	8.8	75.0	methylmalonate	22.4	117.0
propionate	10.2	73.0	malonate	22.8	103.0
formate	11.5	45.0	tartarate	22.8	149.0
butyrate	12.2	87.0	maleate	23.7	115.0
2-keto-D-gluconate	12.2	193.0	a-ketoglutarate	24.6	145.1
2-hydroxyvalerate	12.5	117.1	oxalate	25.3	89.0
pyruvate	12.9	87.0	fumarate	25.6	115.0
isovalerate	13.1	101.1	oxalacetate	28.5	131.0
IStd valerate-d_9	14.0	110.0	citrate	33.5	191.0
valerate	14.3	101.1	IStd citrate-d_4	33.5	195.0
galacturonate	14.4	193.0	isocitrate	34.3	191.0
5-keto-D-gluconate	19.1	193.0	*cis*-aconitate	35.1	173.0
IStd glutarate-d_6	21.5	137.0	*trans*-aconitate	36.7	173.0

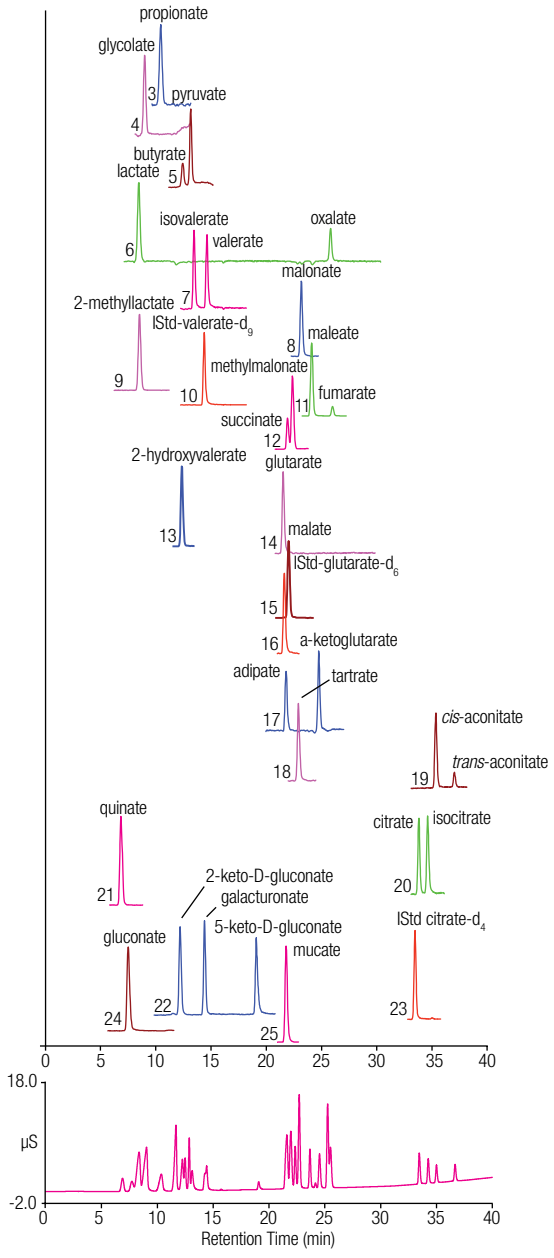


Figure 6-18. CD and MS SIM chromatogram of 32 small organic acids

Results

Organic acids predominantly produce single negatively charged ions $[M-H]^-$ regardless of their valences. $[M-H]^-$ was used to determine and quantify each analyte and selected spectra are seen in Figure 6-19. Cone voltages controlling the extent of in-source CID were optimized for each SIM channel. Conductivity and SIM chromatograms are seen in Figure 6-18.

Quantification can be performed after the generation of respective calibration curves for each analyte. A representative calibration curve of glutaric acid is seen in Figure 6-20 using an isotope-labeled analogue as internal standard.

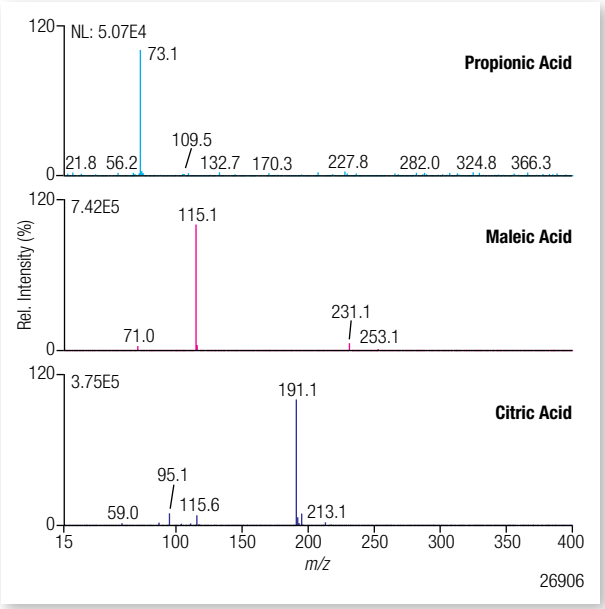


Figure 6-19. MS spectra of propionic acid (monovalent), maleic acid (divalent) and citric acid (trivalent). 25 ng injection for each analyte.

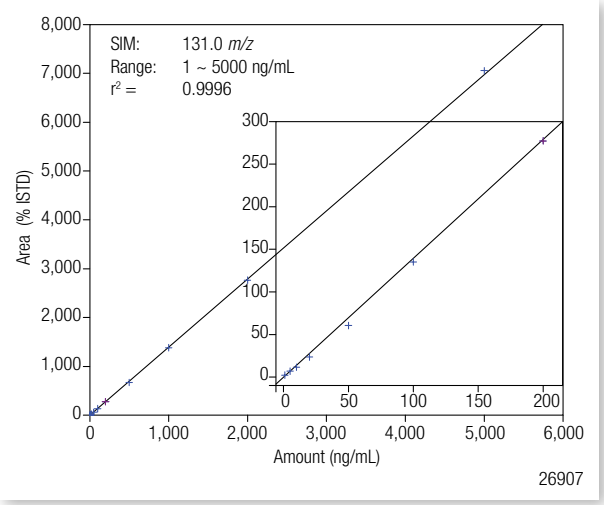


Figure 6-20. Calibration curve of glutaric acid

Ionic Liquids by IC-MS and LC-MS

Introduction

The characteristic composition of ionic liquids (an organic cation or anion and a counterion, in organic or inorganic form) exhibits unique properties, such as extremely low vapor pressure, excellent thermal stability, electrical conductivity, high polarity, and miscibility with various types of solvents. Ionic liquids are organic salts with relatively low melting points (below 100 °C) and have been used as solvents in catalysis, organic chemistry, electrochemistry, and separation science.

Here, two profile methods using IC or LC for the quantitative determination of anionic ionic liquids and anions are presented.

IC Analysis

Chromatographic conditions are shown in Figure 6-21. An MSQ Plus single quadrupole mass spectrometer operated in SIM mode with an ESI interface is used to couple the IC-MS. As seen in Table 6-1, closely eluted analytes can be easily differentiated and are shown as a single peak in each SIM channel. Probe temperature was set at 500 °C; nitrogen was used as the nebulizer gas and set at 85 psi; needle voltage was set at 1.0 kV, and cone voltages were optimized for each SIM scan.

IC Results

Figure 6-21 shows the chromatographic separation of 16 analytes as achieved on a Dionex IonPac AS20 anion-exchange column.

Quantitative experiments provided coefficients of determination (r^2) >0.99 for each analyte in the calibration range from low ppb level to 1000 ppb. Method detection limits were achieved at low ppb level in the range from 1.04 ppb (tosylate) to 6.13 ppb (sulfate). Three commercially available ionic liquids (dissolved in DI water at 2 mg/mL) were analyzed for impurities by this IC-MS method. Eight anions were detected in these ionic liquids at very low ppm levels. Acetate, chloride, bromide, sulfate, and PF_6 were observed in individual or all samples at quantifiable levels (>2.5 ppm in the original sample). Chloride (in two of the three samples) and bromide (in all samples) were observed as the major impurities. (Refer to Dionex [now part of Thermo Scientific] LPN 2369 for details.)

LC Analysis

The study presented here describes the first LC-MS method for the simultaneous analysis of ionic liquids, counterions, and halide ion impurities in a single chromatographic run on an Acclaim Trinity P1 trimode column, using mass spectrometry to ensure selective and sensitive detection. As seen in Figure 6-22, ionic liquids, counterions, and impurities were chromatographically separated on the Acclaim Trinity trimode column. Analytes were eluted in groups in the following order: organic cations, inorganic cations, inorganic anions, and organic anions. The upper traces in Figure 6-22 show the SIM chromatograms of selected analytes, illustrating the specificity and selectivity of the MSQ Plus mass spectrometry detector.

Table 6-1. Ionic Liquids, Counterions, and Impurities In Figure 6-21

Peak	Analyte	Formula	Ret. Time	SIM	Scan Event	Cone Voltage
1	Fluoride	$[F+HF]^-$	4.4	39.0	3.5–7.2	25
2	Acetate	CH_3COO^-	5.0	59.1	3.5–7.2	50
3	Methanesulfonate	$CH_3SO_3^-$	5.3	95.0	3.5–7.2	60
4	Butanesulfonate	$CH_3(CH_2)_3SO_3^-$	6.0	137.2	3.5–7.2	60
5	Chloride	Cl^-	6.5	35.0	3.5–7.2	80
6	Trifluoroacetate	CF_3COO^-	7.9	113.1	7.2–9.5	30
7	Bromide	Br^-	9.0	78.9	8.5–9.5	100
8	Nitrate	NO_3^-	9.9	62.0	9.5–11.4	75
9	Sulfate	HSO_4^-	10.7	97.1	9.5–11.4	50
10	Tosylate	$CH_3C_6H_4SO_3^-$	12.0	171.0	11.4–12.8	60
11	Tetraborate	BF_4^-	13.4	87.0	12.8–15.1	50
12	Triflate	$CF_3SO_3^-$	13.8	149.1	12.8–15.1	60
13	Phosphate	$H_2PO_4^-$	14.3	97.1	12.8–15.1	50
14	Iodide	I^-	15.7	127.0	15.1–17.6	90
15	Thiocyanate	SCN^-	19.4	58.0	17.6–22.6	50
16	Perchlorate	ClO_4^-	20.5	99.0	17.6–22.6	80
17	Hexafluorophosphate	PF_6^-	27.7	145.1	22.6–30.5	80

LC Results

The data presented here demonstrates the unique properties and superior chromatographic performance of the Acclaim Trinity trimode column and its application for simultaneous analysis of ionic liquids, counterions, and impurities. This method can be used for ionic liquid quality assurance, contamination analysis, and the assessment of residues and the effectiveness of any removal process.

Column:	Dionex IonPac AS20 and AG20
Mobile Phase:	Hydroxide gradient

Time (min)	OH
–5	10 mM
0	10
6	30
14	60
16	00
30	100
30.5	10

Flow Rate:	0.25 mL/min
Inj. Volume:	20 µL
Column Temp.:	35 °C
Detection:	A) Suppressed conductivity B) MSQ Plus single quadrupole mass spectrometer

Mass Spectrometric Conditions

Interface:	ESI
Probe Temperature:	500 °C
Nebulizer Gas:	Nitrogen at 85 psi
Needle Voltage:	1.0 kV
Scan Mode:	SIM See Table 6-1 for details.
Injected Amount:	20 ng for suppressed conductivity 4 ng for MS SIM

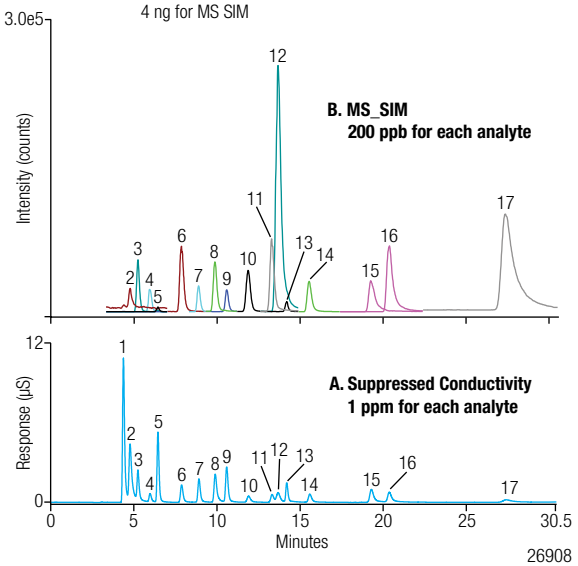


Figure 6-21. Suppressed conductivity and MS SIM chromatograms for ionic liquids and anions

Column:	Acclaim Trinity P1, 2.1 × 100 mm, 3 µm
Mobile Phase:	A) Acetonitrile B) 100 mM ammonium acetate C) DI water;
Gradient:	55% A, 5% B, 40% C from –4 min to 2 min, increase to 60% A, 5% B, 35% C from 2 min to 10 min, increase to 90% A, 5% B, 5% C from 10 min to 11 min, 90% A, 5% B, 5% C from 11 min to 18 min
Flow Rate:	400 µL/min
Inj. Volume:	5 µL
Detector:	MSQ Plus single quadrupole mass spectrometer

Peaks:	
1. Lidocaine	235 *m/z*
2. 1-Butyl-3-methylimidazolium	139
3. 1-Ethyl-3-methylimidazolium	111
4. Sodium (Na+6[CH_3CN])$^+$	269
5. Potassium	39
6. Methanesulfonate	95
7. Tetrafluoroborate	87
8. PF_6^-	145
9. Chloride	35
10. Bromide	81
11. Iodide	27
12. Tosylate	171
13. Docusate	421

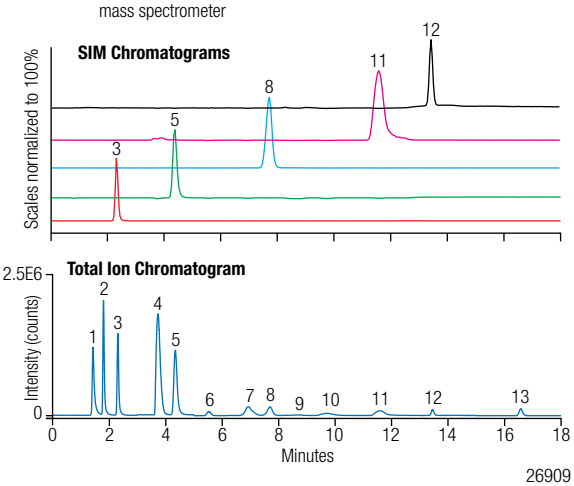


Figure 6-22. LC-MS for the simultaneous analysis of ionic liquids, counterions, and impurities

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