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**ASAP Overview**

**Introduction**

The Waters® Atmospheric Solids Analysis Probe® (ASAP), introduced by McEwan *et. al.*, is a useful tool for the rapid and direct analysis of volatile and semi-volatile, solid, and liquid samples using atmospheric pressure ionization (API). With ASAP, workflow efficiencies are greatly enhanced, saving valuable time and reducing the cost of analyses.

The ASAP technique utilizes the heated nitrogen desolvation gas to vaporize the sample and a corona discharge for sample ionization. This allows low polarity compounds not amenable to ESI to be ionized with a high degree of sensitivity. Furthermore, complex mixtures can be analyzed without the need for any sample preparation, which reduces solvent usage and minimizes environmental impact.

The ASAP is readily fitted to a standard API source by replacing either the ESI or APCI probe assembly. A corona discharge pin also requires fitting. The close proximity of the sample, when loaded onto a glass capillary tube, to the point of ionization and the MS inlet, improves sensitivity while also ensuring safety due to the enclosed source housing.

The probe is designed to have both fixed and removable sections to allow rapid sample introduction without removal of the complete assembly which ensures stable source conditions.

There are two primary mechanisms of ionization in positive ion APCI, as described by Horning 1: Protonation and Charge Transfer.

In the first mechanism, ionization can take place through proton transfer reactions. This example shows the proton source as water, but MeOH or other solvents can be used.

The second mechanism is charge transfer initiated by corona discharge ionization of the nitrogen in the source to generate radical cations of nitrogen. The nitrogen radicals then undergo charge transfer with analyte molecules to generate radical cations of the analyte molecules. Ionization via charge transfer is particularly useful for the analysis of non-polar compounds.

It is possible to select between proton transfer and charge transfer by altering the source conditions, depending on the chemistry of the target analytes or the desire to use either mechanism to discriminate against certain matrix components.

While both of these techniques may be new to many practitioners, ions are formed through well-understood chemical ionization processes. For those familiar with LC/MS, the later mechanism is not available due to the great excess of vaporized solvent present in the API source during LC/MS APCI operation.

**Reference**


*Waters Atmospheric Solids Analysis Probe (ASAP) has been developed under license to M&M Mass Spec Consulting LLC, Hockessin, Delaware (patent pending).*
GOAL
To demonstrate the feasibility of the Atmospheric Solids Analysis Probe (ASAP) in a walk-up environment, organic synthesis reactions were monitored.

BACKGROUND
In organic synthesis labs, reactions are often monitored by thin layer chromatography (TLC), which requires mobile phase optimization. Developing a TLC plate can be a time-consuming process, whereas all chemists need to accomplish is to monitor when the disappearance of the starting materials and the appearance of the final product.

To increase the efficiency of the analysis of these reactions, an open access ASAP system was introduced. With minimal training, chemists were able to analyze their own samples while the analytical chemists concentrated on more difficult compounds.

Without reaction workup or sample preparation, in less than one minute, molecular weight information can be obtained and informed decisions made without delay.

Without reaction workup or sample preparation, in less than one minute, sample information can be obtained and informed decisions made without delay.
Once the ASAP probe was inserted into the sealed MS source enclosure, the desolvation gas was rapidly heated to 400 °C for MS acquisition. MS full scan data from 60 to 2000 amu was acquired for 0.5 min using a 0.2 sec scan duration. Target masses were extracted from the full scan data for reporting. The target masses were for the precursors and the final product.

The report was emailed to the user as a pdf (Figure 3). The report contained a simple table showing whether each target mass was found or not, as well as a spectra showing the found masses. The pdf format aided in copying the results to an electronic lab notebook. This facilitated viewing and sharing of the information.

**THE SOLUTION**

ASAP coupled to an ACQUITY® SQD was used for the analyses. The sampling procedure is shown in Figure 1. First, the sample was loaded onto the sealed glass melting point capillary tube of the ASAP probe by dipping the tip into the sample. The sample information was then entered into the OALogin software (Figure 2), which is a tool with MassLynx™ Software’s OpenLynx™ Open Access Application Manager.

During the login procedure, a user is asked to enter a minimal amount of data. On a single page, the user enters their name and chooses an appropriate method from a predefined list. They then enter sample information, place the probe in the outer assembly, and hit OK.

**SUMMARY**

The ASAP probe can be used in conjunction with a quadrupole mass spectrometer to examine starting materials and monitoring the progress of reactions. Without reaction workup or sample preparation, in less than one minute, sample information can be obtained and informed decisions made without delay. Pairing ASAP with OALogin further simplifies the process and enables anyone to use the system.

As a result, the organic synthesis workflow efficiency is greatly enhanced, saving time and reducing the cost of analysis.
Fine and Specialty Chemicals
AIM
To show the potential of the Waters® Atmospheric Solids Analysis Probe (ASAP) for the rapid identification of impurities in synthesised products followed by further characterization using UPLC®/TOF/MS.

INTRODUCTION
Analytical laboratories studying the products of organic synthesis have to consider many things from confirmation of the final product to identification of impurities. Impurity identification, whether expected or not, is an essential part of the manufacture of fine chemicals, as any impurities could adversely effect the final product. This applies equally to the raw starting materials and the final synthesized product.

For many analytical laboratories, what is required is a rapid verification that the starting materials or synthesized products are the correct materials. At a later stage in the manufacturing process, the detection and identification of impurities becomes more critical. The QC laboratory for the bulk chemical manufacturer is looking to characterize the product for consistency, as well as quality control of reagents and intermediates. The products may have a wide range of chemical properties, placing significant demands on the analytical laboratory on the choice of analytical technique.

This application note introduces a means of obtaining rapid confirmation of the synthesized product, as well as detection of impurities, using the ASAP. This technique allows for rapid, direct analysis of both solid and liquid samples. It is particularly useful for compounds that are non-polar and not normally amenable to analysis by atmospheric pressure ionization techniques.

To fully characterize a sample, the use of additional techniques, such as nuclear magnetic resonance (NMR), infra-red spectroscopy, and LC/MS or GC/MS is required. Here, we show the application of high resolution UPLC/TOF-MS for further characterization of low level impurities.
The sample analyzed was octahydroacridine (>97% purity), a compound of great interest as it plays an important role in the preparation of alkaloids, dyes, drugs, and other biologically active compounds.

**MS system**
Waters LCT Premier™ XE System

**ASAP conditions**
- Ionization mode: ESCi®
- Sample cone: 30 V
- Aperture 1 voltage: 10 V
- Source temp.: 120 °C
- Desolvation temp.: 50 to 450 °C
- Desolvation gas flow: 500 L/hr
- Capillary voltage (ESI): 3000 V
- Corona current (APCI): 5 µA
- Mass range: m/z 50 to 1000
- Scan time: 0.50 s
- Inter-scan delay: 0.02 s
- Lock reference (ESI): Leucine enkephalin, 200 pg/µL at 3 µL/min

The instrument was operated in the combined electrospray/APCI mode (ESCI). This facilitated the acquisition of both analyte data in APCI mode and reference data (for lock mass correction) in electrospray mode.

The sample was loaded by dipping the melting point capillary from the ASAP into the solid sample; excess removed by blowing with a stream of nitrogen.

**UPLC/MS conditions**
- UPLC system: ACQUITY UPLC®
- Column: ACQUITY UPLC BEH C₁₈, 2.1 x 50 mm, 1.7 µm at 35 °C
- Mobile phase A: Water + 0.1% formic acid
- Mobile phase B: Acetonitrile + 0.1% formic acid
- Flow rate: 0.6 mL/min
- Gradient: 10% B (1 min hold) → 25% B at 3 min → 30% B at 4 min
- Injection volume: 10 µL
- Ionization mode: ESI+

Standard electrospray MS conditions were used for the analysis. Data were acquired with aperture 1 voltages up to 60 V to generate in-source CID fragments for structural elucidation.

The sample was dissolved and diluted in methanol to produce a 6 ng/µL solution.
RESULTS AND DISCUSSION

ASAP

The spectrum and elemental composition report from the ASAP analysis of the octahydroacridine solid are shown in Figure 1. The elemental compositions of all ions above 0.5% of the base peak intensity are reported. The spectrum illustrates the expected [M+H]+ ion at m/z 188.1436 (-0.3mDa, -1.6ppm) for the major component as well as a number of low intensity peaks. These low intensity peaks could either be fragments or impurities. No elemental compositions are given for the ions at m/z 189 and 190, as these are the isotope peaks associated with the major component, but all other masses reported have exact mass measurements of <3 ppm.

Figure 1. ASAP spectrum and elemental composition report for octahydroacridine and potential impurities with postulated structures.
**UPLC/TOF/MS**

The BPI chromatogram obtained from UPLC/MS analysis of octahydroacridine implies high compound purity, as shown in Figure 2, dominated by the expected compound along with several minor peaks.

![BPI chromatogram from UPLC/TOF/MS analysis of octahydroacridine.](image1)

Figure 2. BPI chromatogram from UPLC/TOF/MS analysis of octahydroacridine.

In source, collision induced dissociation (CID) fragments can be generated by increasing the aperture 1 voltage, allowing some structural information about the compound to be determined, as shown in Figure 3. This approach confirms the ions at m/z 160 and 186, observed by ASAP, are the result of fragmentation of the major component.

![Spectra for octahydroacridine at aperture 1 voltages of 35 V(A) and 60 V(B) showing increasing fragmentation.](image2)

Figure 3. Spectra for octahydroacridine at aperture 1 voltages of 35 V(A) and 60 V(B) showing increasing fragmentation.
The automated structure elucidation tool, MassFragment™ Software, which uses a systematic bond cleavage and ranking algorithm, was employed to rationalize and identify fragment ion structures from potential impurities. By submitting a postulated precursor structure and a CID high energy spectrum, the MassFragment Software tool was able to generate a report of the possible fragmentation with exact mass confirmation. An extract from the MassFragment Software results from the major component, m/z 188, is illustrated in Figure 4.

Figure 4. MassFragment Software report from the CID spectrum of octahydroacridine.
The MassFragment Software report further confirmed the ions observed at m/z 160 and 186 are fragments of the major component.

The masses detected by ASAP were extracted as exact mass chromatograms (0.02 Da) and are illustrated in Figure 5. The presence of the potential impurities previously identified by ASAP at m/z 184, 186, 198, and 202 were confirmed, along with the fragments at m/z 160 and 186 from the major component.

**CONCLUSIONS**

ASAP was used for the rapid analysis of a fine chemical without any sample preparation or chromatographic separation, enabling rapid confirmation of the synthesized product and detection of trace level impurities.

The synthesized product was further characterized by submitting it to analysis by UPLC/MS. Traditionally, this would have been a time-consuming task, requiring the development of chromatographic conditions to separate the impurities from the major product and interpretation of the MS data to give proposed structures.

The high resolution chromatography associated with UPLC enabled rapid separation of the impurities and, when coupled with the sensitivity and exact mass capabilities of the LCT Premier XE System, made this the ideal tool for impurity profiling.

Mass Fragment Software, a software package designed for the interpretation of fragmentation data, enabled rapid automated interpretation of the in-source CID data generated.

For production plants that require rapid confirmation of starting materials or manufactured product, ASAP, in conjunction with the LCT Premier XE System, provides a simple, rapid, high-resolution, cost-effective technique for the study of synthesized organic compounds. This approach combines the advantages of TOF technology — exact mass information, high energy fragment ions, and reliable isotopic patterns with a quick and easy way to introduce the sample — the Atmospheric Solid Analysis Probe.

When further analysis is required for the identification of unexpected impurities, whether as a result of manufacturing, contamination, or degradation, the enhanced resolution associated with UPLC facilitates rapid separation of these impurities from the expected product.

MassFragment Software, a software package specifically designed to facilitate the interpretation of fragmentation data, completes the solution.
Improving Organic Synthesis Reaction Monitoring with Rapid Ambient Sampling Mass Spectrometry

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INTRODUCTION

To maximize return on investment, the chemical industry is challenged to bring new products to the market while improving productivity. In many cases, developing a new product or a new process with only a small percentage increase in the yield can result in significant increases in earnings for the company. To develop new chemicals and improve organic synthesis efficiency, timely chemical analysis data on the progress of reactions and the product purification processes are required.

In organic synthesis labs, reactions are often monitored by TLC, which does not provide chemical structure information and requires mobile phase optimization. Conventional analytical instruments such as NMR, LC, or LC/MS require time-consuming reaction workup procedures including extraction, filtration, separation, and evaporation. Frequently, it takes hours to obtain the critical chemical structure data. Moreover, when analyzing new reactions and new compounds, method development and expert data interpretation are needed. Therefore, technologies that can quickly provide structural information are highly desirable.

The Waters® Atmospheric Solids Analysis Probe (ASAP) is an ambient desorption ionization technique for mass spectrometric (MS) analysis and is capable of ionizing a wide range of volatile and semi-volatile analytes. The ionization mechanism is similar to atmospheric pressure chemical ionization (APCI). The technique uses heated nitrogen desolvation gas to vaporize the sample and corona discharge for ionization. Solid and liquid samples are directly loaded on to ASAP for MS acquisition, as shown in Figure 1.

APPLICATION BENEFITS

Without reaction workup or sample preparation, and in less than one minute, structural information can be obtained and informed decisions made without delay. As a result, the organic synthesis workflow efficiency is greatly enhanced, saving time and reducing the cost of analysis.

Figure 1. The sample is loaded directly onto the tip of a glass capillary. The sample is then directly inserted into the ionization source chamber. Bulk MS data are collected in seconds.

WATERS SOLUTIONS
Atmospheric Solids Analysis Probe (ASAP)
ACQUITY® TQD
PoraPak® Rxn CX Cartridge

KEYWORDS
Organic synthesis, volatile and semi-volatile analytes, ionization technique, reductive amination, amide formation reactions, solid-phase extraction fractions
This application note demonstrates the use of ASAP, in conjunction with a tandem quadrupole mass spectrometer (ACQUITY TQ Detector) for examining starting materials, monitoring the progress of reductive amination and amide formation reactions, and identifying components in solid-phase extraction fractions. Without reaction workup or sample preparation, in less than one minute, structural information can be obtained and informed decisions made without delay. As a result, the organic synthesis workflow efficiency is greatly enhanced, saving time, and reducing the cost of analysis.

**EXPERIMENTAL**

**Reductive amination**  
(Scheme 1)  
Acetophenone (0.218 g, 1.8 mmol) and aniline (0.169 g, 1.8 mmol) were added to a glass vial containing 3.5 mL of methanol. To the vial, 1.2 mL of 1.5 N HCl in methanol was added. The mixture was stirred at room temperature for two minutes, and then 10 mL of a 11.3 mg/mL solution (1.8 mmol) of NaBH₃CN in methanol was added to start the reaction. The progress of the reaction was monitored using ASAP coupled to the ACQUITY TQD.

**Acylation reaction with acetylimidazole**  
(Scheme 2)  
Acetylimidazole (416 mg, 3.78 mmol) was dissolved in 5 mL of acetonitrile. Phenylphenylamine (170 mg, 1 mmol) was dissolved in 5 mL of acetonitrile in a second vial. The two solutions were then mixed and the reaction progress was examined with ASAP, coupled with the ACQUITY TQD.

**Acylation reaction with acetic anhydride**  
(Scheme 3)  
To a glass vial containing 10 mL of acetonitrile, 4-dimethylaminopyridine (0.257 mg, 2.1 mmol) and methoxybenzylamine (285 mg, 2.1 mmol) were added and the mixture was stirred at room temperature for one minute. An excess amount of acetic anhydride (320 mg, 3.1 mmol) was added to start the acylation reaction. Upon completion of the reaction, 8 mL of the mixture was gravity loaded on to a Waters 20 mL PoraPak™ Rxn CX Cartridge. The product fraction was eluted with 10 mL of methanol, and the base catalyst (4-dimethylaminopyridine) was eluted with 20 mL of 5% ammonium hydroxide in methanol.

**ASAP analysis method**  
The ASAP coupled the ACQUITY TQD was used for the analyses. The sampling procedure is shown in Figure 1. First, the sample was loaded onto the sealed glass melting point capillary tube of the ASAP probe by dipping the tip into the reaction mixture. The ASAP probe was then inserted into the sealed MS source enclosure and the desolvation gas was rapidly heated to 400 °C for MS acquisition. MS full scan data from 60 to 500 amu were acquired for 0.5 min using a 0.2 sec scan duration with the following MS tune page parameters:

- Ionization mode: ESI positive
- Corona current (APcI): 5 µA
- Cone voltage: 2 V
- Extractor: 1 V
- Desolvation temp.: 400 °C
- Source temp.: 120 °C
- Desolvation gas: 500 L/Hr
- Cone gas: 0 L/Hr
- Collision gas flow: 0.1 mL/min
To avoid in-source fragmentation and pyrolysis, a low cone voltage (2 V) and low desolvation temperature (400 °C) were applied. To minimize the formation of radical cations (M+), a 2 mL glass vial filled with water was placed inside the source chamber. As a result, highly reproducible mass spectra were obtained. Prior to each analysis, a new glass capillary tube was inserted into the source and baked for approximately 15 sec to minimize any background ions. The acquisition was initiated while the newly baked capillary was still in the source to obtain background reference scans. The probe was removed from the source and sample was applied. The probe was then re-inserted while acquisition continued. Combined mass spectra were obtained by subtracting the baseline of the reference scans from the total ion current profiles of the samples.

RESULTS AND DISCUSSION

Reductive amination reactions are widely used in multiple-step organic syntheses for making agricultural chemicals, food additives, drugs, and building blocks for high performance materials. A reductive amination reaction for making phenyl(phenylethyl)amine (Scheme 1) was conducted using the same conditions reported by Borch et al.\(^1\) The reaction mixture could not be analyzed by LC or LC/MS techniques without workup procedures to remove NaBH\(_3\)CN since the reducing agent would plug ESI capillary tubes and damage LC columns. In contrast, using ASAP, the reaction mixture was directly loaded onto the melting point capillary of the probe for MS analysis without removing the reducing agent.

![Scheme 1. Reductive amination for making phenyl(phenylethyl)amine.](image)

<table>
<thead>
<tr>
<th>MS TUNE PAGE PARAMETERS</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionization mode:</td>
<td>ESI+</td>
</tr>
<tr>
<td>Corona current (APcI):</td>
<td>5 μA</td>
</tr>
<tr>
<td>Cone voltage:</td>
<td>2 V</td>
</tr>
<tr>
<td>Extractor:</td>
<td>1 V</td>
</tr>
<tr>
<td>Desolvation temp.:</td>
<td>400 °C</td>
</tr>
<tr>
<td>Source temp.:</td>
<td>120 °C</td>
</tr>
<tr>
<td>Desolvation gas:</td>
<td>500 L/Hr</td>
</tr>
<tr>
<td>Cone gas:</td>
<td>0 L/Hr</td>
</tr>
<tr>
<td>Collision gas flow:</td>
<td>0.1 mL/min</td>
</tr>
</tbody>
</table>
Figure 2 shows the full scan mass spectra of step-by-step reaction procedures. Spectrum 2a was acquired after mixing acetophenone (AP) and aniline (AN) in methanol. Four major ion peaks, protonated aniline ion (m/z 94), protonated acetophenone ion (m/z 121), adduct ion of aniline and acetophenone (m/z 214), and dimer ion of acetophenone (m/z 241) were observed. Their identities were confirmed using MS/MS (data not shown). 2 min after adding HCl solution, the formation of an iminium intermediate4 (IMM) with m/z 196 was apparent in the mass spectrum (Figure 2b). In Figure 2c, acquired 10 min after adding the reducing agent, the spectrum revealed the formation of a protonated phenyl(phenylethyl)amine ion (m/z 198) and the disappearance of the iminium intermediate. The increase in relative ion intensity of the product and decrease in that of the reactant ions after 40 min are shown in Figure 2d. Finally, in the spectrum acquired after 4 hours, no remaining reactants were visible (Figure 2e).

Using ASAP with the ACQUITY TQD, product ion spectra of targeted ions in reaction mixtures can be easily acquired to further confirm the identity of the ions detected in full scan mode. Figure 3 shows the product ion spectrum of m/z 198 acquired at a collision energy of 7 V. Two product ion peaks with m/z of 94 and 105 were observed, confirming that m/z 198 is indeed the protonated phenyl(phenylethyl)amine ion. The experiments demonstrate that without reaction workup or sample cleanup, ASAP rapidly provides structural information on the progress of reductive amination reactions to maximize organic synthesis efficiency and yields.

Figure 2. ASAP full scan mass spectra for monitoring reductive amination: 2a, acquired after mixing acetophenone (AP) and aniline (AN) in methanol; 2b, acquired 2 min after adding HCl solution; 2c, acquired 10 min after adding NaBH₃CN; 2d, acquired 40 min after adding NaBH₃CN; 2e, acquired 4 hours after adding NaBH₃CN.

Figure 3. Product ion spectrum of m/z 198: confirming the structure of the targeted synthesis reaction product, phenyl(phenylethyl)amine (P).
Amide bond formation through acylation reactions is another synthetic pathway of critical importance in many industries. The rate of acylation reactions depends highly on the amines, acylation reagents and catalysts used in the reaction. The workup procedures usually include liquid-liquid extraction and evaporation. The products are often purified using flash chromatography or preparative LC. Scheme 2 is an example of acetamide formation using acetylimidazole (AI) as the acylation reagent to yield 4-(phenylphenyl)-acetamide (PPA).

Figure 4 shows the acylation reaction monitored using ASAP with ACQUITY TQD. Spectrum 4a was acquired after dissolving acetylimidazole (AI) in acetonitrile. The molecular ion and dimer ion of acetylimidazole are apparent at m/z 111 and m/z 221, respectively. The observation of imidazole ions (m/z 137, m/z 69) indicates that some acetylimidazole had decomposed. Since acetylimidazole can be hydrolyzed to form imidazole upon exposure to moisture in the atmosphere, its decomposition was not unexpected. Spectrum 4b was acquired after dissolving phenylphenylamine (PA) in acetonitrile. Only protonated molecular ions of 4-phenylphenylamine (m/z 170) and the dimer ion of 4-phenylphenylamine (m/z 339) were observed.

The spectrum in Figure 4c was acquired 10 min after mixing the solutions of AI and PA. Four new ions (m/z 212, 280, 381, and 423) were observed. MS/MS experiments (data not shown) were consistent with the structures labelled in Figure 4c. Three hours after mixing the solutions of acetylimidazole and 4-phenylphenylamine Figure 4d) the AI/IM adduct (m/z 179) was not detected and after 3 days (Figure 4e) the limiting reagent (PA) was no longer evident.

Figure 4. ASAP full scan mass spectra for monitoring acylation: 4a, acquired after dissolving acetylimidazole (AI) in acetonitrile (ACN); 4b, acquired after dissolving phenylphenylamine (PA) in acetonitrile; 4c, acquired 10 min after mixing the solutions of AI and PA; 4d, acquired 3 hours after mixing the solutions of AI and PA; 4e, acquired 3 days after mixing the solutions of AI and PA.
Scheme 3 shows another example of acetamide formation reaction using acetic anhydride as the acylation reagent and 4-dimethylaminopyridine (DMAP) as the base catalyst to produce N-[(4-methoxyphenyl)methyl] acetamide (MAA).

Figure 5 shows the full scan mass spectra of the step-by-step reaction and separation procedures of the acylation reaction. Spectrum 5a was acquired after mixing methyloxybenzylamine and the base catalyst DMAP in CH3CN. Four major ion peaks from the starting material and the catalyst were identified, as shown in Figure 5a. 5 min after adding an excess amount of acetic anhydride, ions from the formation of the protonated N-[(4-methoxyphenyl) methyl] -acetamide ion (m/z 180) and its dimer (m/z 359) were apparent (Figure 5b) and the reactant peaks had disappeared. The results suggest that the reaction was completed in five min.

Figure 5. ASAP full scan mass spectra for monitoring the acylation reaction with acetic anhydride. 5a, acquired after mixing methyloxybenzylamine (A) and the base catalyst DMAP (C) in CH3CN; 5b, acquired 5 min after adding acetic anhydride; 5c, the product fraction eluted with 100% MeOH from a PoraPak Rxn CX Cartridge; 5d, the fraction eluted with MeOH solution containing 5% of ammonia hydroxide.
The reaction mixture was purified with a PoraPak Rxn CX Cartridge to separate the product N-[(4-methoxyphenyl) methyl]acetamide (MAA) from the catalyst DMAP. The PoraPak Rxn CX Cartridge contains strong cation-exchange sorbents designed to separate neutral and base compounds without conventional workup procedures. The reaction mixture was first loaded onto a 20 mL PoraPak Rxn CX Cartridge. Then, 100% methanol was used to elute the product, MAA. Figure 5c shows the spectrum of the methanol eluted fraction and only the protonated N-[(4-methoxyphenyl) methyl]acetamide ion ($m/z$ 180) and its dimer ion ($m/z$ 359) were observed. The trapped base compounds were eluted from the cartridge using a methanol solution containing 5% ammonium hydroxide. Only the protonated DMAP ion ($m/z$ 123) and the dimer ion ($m/z$ 245) were observed, as shown in Spectrum 5d.

The data illustrate that the reaction product MAA was successfully separated from DMAP with the PoraPak Rxn CX Cartridge.

As demonstrated in this work, ASAP coupled to an ACQUITY TQ Detector can provide full scan MS, as well as MS/MS experiments. The system can rapidly examine the progress of reactions, confirm the formation of reaction products, identify product fractions from solid phase extraction, and obtain product ion spectra for structural confirmation. An alternative option for rapid testing of simple mixtures is the single-stage quadrupole mass spectrometer (ASAP/ACQUITY SQD), which offers ease-of-use and a lower cost. For comprehensive sample characterization, ASAP coupled to a quadrupole time-of-flight mass spectrometer, such as the Xevo® G2 Q-Tof provides high sensitivity, full spectrum analyses, accurate mass data, and the ability to perform MS/MS experiments. Depending on the complexity of the reaction mixtures and the testing requirements, each of these systems offers different benefits.
CONCLUSIONS

This application note demonstrates the benefits of ASAP for organic synthesis labs. Without workup or separation, ASAP together with MS can be used to directly analyze reaction mixtures. Mass spectra can be obtained in less than one minute to:

- Identify the major components in starting materials
- Monitor the progress of reactions
- Confirm the formation of reaction products
- Compare the relative abundance of products
- Identify product fractions during reaction workup and flash chromatography separation

In this way, informed decisions can be made without delay, greatly enhancing the workflow efficiency of organic synthesis. The ASAP solution reduces solvent usage and lessens the impact on the environment in line with the principles of green chemistry. As a result, daily lab operating costs can be reduced.

References

Rapid Analysis of Complex Heterogeneous Mixtures for Active Components in Cosmetic Products

GOAL
To employ Waters® Atmospheric Solids Analysis Probe (ASAP) as a sample inlet for mass spectrometry to directly analyze heterogeneous samples and eliminate sample preparation.

BACKGROUND
Active components, such as UV absorbers are widely used in formulated personal care products, including sun block and over-the-counter facial creams. These components are constantly under examination for quality control, thermal stability or shelf life, and photochemical stability. The sample matrix is a heterogeneous complex mixture that includes components as diverse as pigments, oils, emulsions, and functional or active organic chemicals. Typical analyses of these types of materials include the use of conventional analytical tools, such as NMR, LC, or LC/MS. These techniques require time-consuming workup procedures that include precipitation, extraction, filtration, separation, and evaporation. ASAP can provide mass spectra of mixtures within seconds without sample workup, which streamlines the workflow when monitoring mixtures in targeted analysis.

Streamline your workflow when monitoring mixtures in targeted analyses with ASAP, which provides mass spectra of mixtures within seconds – no sample workup required.

Figure 1. The complex sample spectra were recorded for the bulk sample without dilution, extraction, or any sample pre-treatment. Note that both negative and positive ionization were collected – positive ionization data are displayed.
Analysis of active ingredients in personal care products was routinely conducted using ASAP as a mass spectrometer inlet with a total analysis time of two minutes. This technique allowed for direct sample introduction, without the need for sample preparation. Complex sample matrix interferences were easily addressed with a targeted analysis using established fragmentation patterns produced in the collision cell, which resulted in unique analyte detection.

**SUMMARY**

- Using ASAP as a sample inlet for analysis of heterogeneous sample matrices allows for collection of characteristic mass spectra and analysis of relative concentration of components in a product mixture.
- This analytical approach can be used to monitor key ingredients, as well as to profile product integrity.
- ASAP can provide critical data and increased analysis capacity with minimal specialized operator training required to support researchers, as well as production operations.

**THE SOLUTION**

A tandem quadrupole mass spectrometer with an ASAP probe was used to analyze critical components in formulated products, without the need for extensive sample preparation or isolation of the analyte from a heterogeneous matrix. The sample was loaded onto a glass tube on the probe by dipping its tip directly into the product mixture. The probe was inserted into the MS source at atmospheric pressure. Desolvation gas heated to 350 °C was used to volatilize the analytes. Mass spectra were acquired in two minutes using both APCi positive and negative mass scan modes. The targeted analytes were isolated from matrix interference based on confirmatory fragmentation.

A complex data set is displayed in the collected spectra, shown in Figure 1, which provide a summation of information from a wide array of product components. Utilizing a targeted workflow approach, an evaluation of the data set focused on expected and other typical components in the various products.

Employing a wide array of collision conditions for the target analyte list, the sample components were analyzed to determine appropriate fragmentation conditions.

Based on the collision cell conditions for each component in the target analyte list, a schedule of MRM scans was established. The samples were then reanalyzed using the ASAP sampling method, and the resulting thermal desorption chromatograms were collected, as shown in Figure 2.

![Figure 2. Thermal desorption chromatograms for the target analyte list using MS/MS data. Each thermal chromatogram was processed using a simple smoothing function to provide consistent data over the acquisition range.](image-url)
Rapid Polymer Analysis with Atmospheric Solids Analysis Probe/ Mass Spectrometer (ASAP/MS)

GOAL
To successfully and rapidly analyze short chain natural and synthetic specialty polymer, surfactant, and oligomeric materials in order to provide absolute molecular weight profiles, data, and valuable material architecture in five minutes or less using desorption mass spectrometry with Waters® ASAP sample inlet and ACQUITY® SQ Detector (SQD).

BACKGROUND
Analysis of specialty polymers and surfactants is often limited to size-based analysis, such as Size Exclusion Chromatography (SEC) with an appropriate detection method. Inherent in this technique is the requirement for a suitable calibration protocol that takes into account detector bias and chromatographic stability. Further, as product space expands to include multi-functional materials, the SEC approach is limited when addressing compositional variation in the material.

The goal of matching the SEC analysis with mass spectrometry can provide necessary compositional analysis, but it is fraught with significant challenges. Typical SEC solvents, such as THF, DMF, and toluene, do not allow for a suitable environment for mass spectral analysis. Infusion of polymeric material for mass spectral analysis has been explored, but this approach is limited due to ionization suppression effects caused by the competing ionization of many infusion solvents. Use of ASAP as a sample inlet provides for direct mass spectral analysis.

ASAP/MS provides data including absolute molecular weight profiles for polymer materials in less than five minutes.

Figure 1. Using ASAP as a sample inlet, a sample is loaded directly onto the capillary tip of the probe, vaporized using heated gas, and then ionized using a corona discharge.
SUMMARY

The versatility and advantages of Waters ASAP/SQD approach has shown that a broad array of samples can be evaluated in one or two minutes, depending on the sample type and its volatility. Reproducible data can be easily obtained without sample specific method development. Further, the unique mass spectral signature of the sample allows for the analysis of compositional ‘fingerprint’ variations not seen with conventional size exclusion separation analysis. The approach offers a reduction in the time required for analysis and operator training, as well as elimination of costs associated with solvent consumption and waste treatment.

THE SOLUTION

ASAP coupled to ACQUITY SQD has proven to be a powerful laboratory tool for polymeric analysis. The utility of the solids probe provides a simple, direct, and rapid mode of sample introduction. Due to sample desorption from the probe tip, the analyte is introduced without interference from solvents, allowing consistent ionization of the analyte. The resulting thermally desorbed molecular chains are ionized across their molecular weight distribution.

The analysis is completed in a few steps:

- The sample is dissolved in solvent and applied to the tip of a melting point capillary tube. The solvent is flashed off of the tip in the first seconds of the analysis due to the controlled desolvation gas flow and temperature. The analyte is left on the capillary tip free of background solvent and related ionization and suppression effects.
- The polymeric material thermally desorbs or volatilize from the tip under controlled desolvation gas temperature and flow.
- As the analyte molecules volatilize they are ionized.
- The ionized molecules are detected using the ACQUITY SQD.

The resulting thermal desorption data is tabulated based on the m/z (equal to mass for singly charged molecules) and abundance of each polymer chain length. The mass is adjusted for proton inclusion and the adjusted mass and abundance data is combined and summed over the weight distribution.

The solution:

ASAP coupled to ACQUITY SQD has proven to be a powerful laboratory tool for polymeric analysis. The utility of the solids probe provides a simple, direct, and rapid mode of sample introduction. Due to sample desorption from the probe tip, the analyte is introduced without interference from solvents, allowing consistent ionization of the analyte. The resulting thermally desorbed molecular chains are ionized across their molecular weight distribution.

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- The ionized molecules are detected using the ACQUITY SQD.

The resulting thermal desorption data is tabulated based on the m/z (equal to mass for singly charged molecules) and abundance of each polymer chain length. The mass is adjusted for proton inclusion and the adjusted mass and abundance data is combined and summed over the weight distribution.

Sample: CH₃(CH₂)nO(CH₂CH₂O)ₙOH

Where m = 10-14 and n = 1-14
Applying ASAP to the Analysis of Synthetic Polymers

GOAL
To provide a direct analysis tool for mass spectrometric analysis of synthetic polymers and blends with limited method development or sample preparation.

BACKGROUND
Analysis of specialty polymers and surfactants is often limited to size-based analysis, such as Size Exclusion Chromatography (SEC) with an appropriate detection mode. When employing this technique, a proper test method must be established, including a calibration set that takes into account detector bias and chromatographic stability. Further, as product space expands to include multi-functional materials, this analytical approach has been found to be limited when addressing compositional variations in the material.

ASAP (Atmospheric Solids Analysis Probe) developed by McEwen et al. has been shown to be a useful tool for the rapid direct analysis of volatile and semi-volatile solid and liquid samples, such as synthetic polymers and oligomers. The ability of Ion Mobility Spectrometry (IMS) to separate ions based on their collision cross sectional area and charge state provides a powerful orthogonal separation technique, when coupled with mass spectrometry for the analysis of complex mixtures.

ASAP IMS-MS shows excellent potential for the rapid fingerprinting of complex polymeric samples.

Figure 1. The sample is loaded directly onto the tip of a glass capillary. The sample is then directly inserted into the ionization source chamber. Bulk MS data are collected in seconds.
**THE SOLUTION**

All analyses were performed using a Waters® SYNAPT® G2 HDMS™ System. An ASAP device was used in place of the instrument’s Electrospray probe, as shown in Figure 1. The source was operated in ESCi® mode to facilitate the use of the Electrospray desolvation heater in conjunction with a corona discharge. This configuration also allowed the LockSpray™ interface to be used for exact mass measurements.

Samples were introduced on a sealed glass melting point tube and vaporized in a stream of heated nitrogen. The temperature of the nitrogen was ramped to control the vaporization of components in the complex mixtures. The sample in the gas phase was ionized by proximity to a corona discharge needle. Ions then passed from the atmospheric pressure region into the mass spectrometer.

The polymer mixture was analyzed by ASAP on a SYNAPT G2 HDMS System, shown in Figure 2, and the IMS-MS data were post-processed using a 3-dimensional peak detection algorithm ‘APEX 3D’ to determine m/z, drift time (DT), and intensity, as shown in Figure 3. Ion mobility separated spectra of the polyether glycol and polystyrene were readily extracted using this software. This approach has potential for wider application in the rapid characterization of polymeric mixtures.

**SUMMARY**

- ASAP provides a rapid method for the direct analysis of complex mixtures such as blended polymers without any sample preparation.
- Non-polar compounds which are not amenable to analysis by ESI or APCI were readily detected with good sensitivity.
- ASAP IMS-MS shows excellent potential for the rapid fingerprinting of complex polymeric samples.

**References**

ASAP-IMS-MS shows the potential to fingerprint crude oil samples, and offers a route to the analysis of involatiles, which cannot be achieved using GC/MS.
THE SOLUTION

Direct sample introduction of a crude oil sample and ionization was performed using the ASAP technique with the SYNAPT G2 HDMS Mass Spectrometer. The conventional ToF-MS spectrum obtained is extremely complex, as shown in Figure 1.

When using ion mobility separation, bands separated by 14 Da (CH2) are visualized, as shown in Figure 2A. By selecting the bands shown in Figure 2B, it is possible to extract the ion mobility mass spectrum and export it into MassLynx™ Software for further interpretation, as shown in Figure 3.

Ion mobility separation (IMS) combined with direct ionization using the ASAP has previously been illustrated. The orthogonality of IMS acts as an enabling technology where crude oil sample analysis can be performed with no prior chromatographic separation or sample preparation. The combination of ASAP-IMS-MS shows the potential for this technique to fingerprint crude oil samples, and offers a route to the analysis of involatiles, which cannot be achieved using GC/MS. Useful information was readily extracted from complex data using DriftScope™ Mobility Environment Software v.2.1.

SUMMARY

- ASAP provides an easy and quick means to introduce a sample and produce screening data, without the constraints caused either by chromatographic conditions, or by ionization solvent compatibility.
- Ion mobility brings an additional dimension to the analysis of complex samples such as crude oil.
- The potential to separate isomers can simplify the mass spectra, and facilitates the characterization of complex samples.
- IMS-MS extends the capability of direct ionization techniques.

References


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GOAL

To provide a system for direct sample introduction of lubricating oil formulations into a mass spectrometer for material characterization without sample preparation.

BACKGROUND

The analysis of complex mixtures in multi-component matrices is a recurring requirement for industrial mass spectrometry laboratories. The full analysis of particularly complex samples may require a strategy that involves several techniques, such as GC/MS and LC/MS. Typical of this type of challenge is the analysis of lubricant formulations. These formulations generally consist of a mineral oil base with anti-oxidants and other performance additives. An oil/additives package of this type contains compounds of widely differing volatility and polarity that provide a significant challenge to analysts.

ASAP (Atmospheric Solids Analysis Probe) developed by McEwen et al. is a useful tool for the rapid and direct analysis of volatile and semi-volatile solid and liquid samples using atmospheric pressure ionization. The ASAP technique is capable of ionizing low polarity compounds not amenable to Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) with high sensitivity. It can also be used for the analysis of complex samples without the need for any sample preparation. The use of ASAP as a sample inlet for Time-of-Flight mass spectrometry (ToF MS) allows for the analysis of complex mixtures, such as lubricating oil, to determine the exact mass of the functional additives.

The ability of ASAP to generate ions by both charge transfer and proton transfer has enabled the ionization of a wide range of compounds of varying polarity.
**SUMMARY**

- The use of ASAP for direct sample introduction allows for component characterization without the need for development of multiple GC and LC separation methods.
- The ability of ASAP to generate ions by both charge transfer and proton transfer has enabled the ionization of a wide range of compounds of varying polarity.
- The ionization of the additives in the lubricating oil formulations was enhanced by addition of methanol to the source atmosphere.
- ASAP ToF MS provides a rapid method for the direct analysis of complex mixtures without any sample preparation, yielding exact mass identification as well as the opportunity to evaluate product degradation.

**References**


**THE SOLUTION**

An ASAP inlet was used to introduce samples into a Waters® SYNAPT® G2 HDMS™ System. Samples were loaded directly onto a sealed glass melting point tube without any sample preparation and vaporized in a stream of heated nitrogen. The sample in the gas phase was ionized in proximity to a corona discharge needle. Ions were then passed from the atmospheric pressure region into the mass spectrometer. Use of APCI allows for evaluation of chemical modifiers, such as methanol, to influence the ionization mechanism, as shown in Figure 1.

This example shows the ASAP analysis of a lubricating oil before and after the addition of methanol to the source region. The addition of the methanol clearly shows the enhancement of the amine antioxidants, which have a higher proton affinity, resulting in the formation of the protonated molecules. The dry nitrogen atmosphere favors the formation of radical cations of the mineral oil by charge transfer. The peak at $m/z$ 421 (M+) in the dry nitrogen atmosphere and $m/z$ 422 ([M+H]+) in the methanol atmosphere are di-nonyl diphenylamine, a commonly used antioxidant. Although the di-nonyl diphenylamine can also be observed as a radical cation at $m/z$ 421 in the spectrum obtained under dry nitrogen atmosphere conditions, the introduction of the chemical modifier allows for analyte discrimination.

The elemental composition report for the additives apparent in the methanol atmosphere spectrum is shown in Figure 2. The results indicate the presence of trimethylolpropane (TMP) ester additives at $m/z$ 369 and 397, while the peaks at $m/z$ 296 and 422 are mono and di-nonyl diphenylamine respectively. These are commonly used antioxidants.

![Figure 2. Exact mass analysis of ester and amine additives in lubricating oil.](image-url)
Food Testing
AIM
To successfully profile flavor ingredients of food products using a simple, rapid technique without sample extraction, filtration, dilution, and chromatographic separation.

BACKGROUND
Flavor that entices all our senses, including olfaction, taste, and texture sensation is one of the paramount factors contributing to successful food products. Each food product manufacturer creates and maintains its own flavor profiles to distinguish its products from competitors. Flavor ingredient profiles differ among food products. To ensure product quality and accurate labeling, the flavor ingredients and the final products are analyzed and authenticated through chemical analysis. However, most chemical composition analysis methods require sample preparation procedures, including extraction, filtration, dilution, and chromatographic separation which are time-consuming and laborious. Therefore, it is most desirable to have a screening tool to quickly examine flavor profiles and authenticate product quality.

ASAP can fingerprint the chemical composition of flavor ingredients in various beverages and food products in less than 3 minutes.

THE SOLUTION
The Waters® Atmospheric Solids Analysis Probe (ASAP), together with a quadrupole mass spectrometer are able to meet the demand. Without sample extraction, sample dilution, and chromatographic separation, ASAP can be used to rapidly fingerprint the chemical composition of flavor ingredients in various beverages and food products, such as vanilla extract, coffee, ice cream, and cookies.

Cookie and ice cream samples were loaded onto the sealed glass melting point capillary tube of the ASAP probe by running the tube directly across the sample surface. The vanilla extract and coffee samples were loaded onto the ASAP probe by dipping the tip of the sealed glass melting point capillary tube into the samples. The ASAP probe was inserted into the sealed source enclosure and the desolvation gas was rapidly heated to 200 °C.
The data in Figures 1 to 4 were acquired using ESI positive mass scan mode at 15-V cone voltage with a 3-minute runtime. Combined mass spectra were obtained by subtracting the baseline of the reference scans from the total ion current profiles of the samples.

Figures 1 to 4 show the mass spectra comparisons between imitation and pure vanilla extracts; French vanilla-flavored coffee and Irish cream-flavored coffee; two cookie samples A and B; and two ice cream samples, A and B. The data show the differences in flavor profiles among these products.

**SUMMARY**

Without sample extraction, sample dilution, and chromatographic separation, ASAP can be used to rapidly fingerprint flavor profiles of various food products and authenticate product quality. Without sample preparation and solvent usage, this 3-minute screening solution has great potential to increase lab productivity through analytical time savings. It can also lessen the impact on the environment, which is consistent with the principles of green chemistry. The end result is a reduction in daily lab operating costs.

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**Figures 1 to 4.** 1. Mass spectra of imitation and pure vanilla extracts 2. French vanilla and Irish cream coffee samples, 3. Two different brands of cookies, 4. Two different brands of ice cream samples.
AIM
To successfully screen for the presence of coumarin in vanilla extracts and food products using a simple, rapid technique without sample extraction, filtration, dilution, or separation.

BACKGROUND
Coumarin is a natural compound found in many plants including tonka bean, woodruff, and certain species of cinnamon. It is often added to counterfeit vanilla extracts in order to increase the vanilla flavor perception. Since coumarin is toxic, it is banned as a food additive in the United States, while in the EU, the maximum tolerance limit for coumarin in food is 2 mg/kg (EC Directive 88/388/ECC). Recently published reports indicate that cases of elevated coumarin levels in Christmas biscuits, gingerbread, cookies, and other food products have increased in the EU market. For regulatory compliance, food companies analyze vanilla extract as well as food products to ensure product quality and accurate labeling. Liquid chromatography with mass spectrometric detection (LC/MS/MS) is the most frequently used technique for analyzing coumarin in food products. Like most analytical techniques, it requires sample preparation procedures including dilution, filtration, and extraction which are time-consuming and laborious. Therefore, it is most desirable to have a screening tool to quickly authenticate product quality.

The ASAP/TQD System can detect the presence of coumarin at 2 mg/kg level in various food matrices in less than 3 minutes.

THE APPROACH
The Waters® Atmospheric Solids Analysis Probe, in conjunction with the ACQUITY® TQ Detector (ASAP/TQD) is a desirable screening tool. Without sample extraction, sample dilution, and chromatographic separation, the ASAP/TQD solution can rapidly detect the presence of coumarin at levels relevant to legislation in various food matrices, including vanilla extract, coffee, ice cream, and cookies.

Cookie and ice cream samples were loaded onto the sealed glass melting point capillary tube of the ASAP probe by running the tube directly across the sample surface. The vanilla extract and coffee samples were loaded onto the ASAP probe by dipping the tip of the sealed glass melting point capillary tube into the samples.
The ASAP probe was inserted into the sealed source enclosure and the desolvation gas was rapidly heated to 200 °C. The data were acquired using multiple reaction monitoring (MRM) mode with the MS parameters listed in Table 1.

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<th>Precursor ion</th>
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<th>Cone voltage (V)</th>
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Table 1. MRM transitions for coumarin.

The MS/MS ion trace profiles from four food samples spiked with coumarin at the legislated level of 2 mg/kg (black traces) versus blank samples (red traces) are shown in Figure 1.

SUMMARY

Without sample extraction, sample dilution, and chromatographic separation, the ASAP/TQD System is able to detect the presence of coumarin at 2 mg/kg level in various food matrices in less than 3 minutes. Without sample preparation and solvent usage, this rapid screening solution greatly increases lab productivity through analytical time savings and also lessens the impact on the environment, which is consistent with the principles of green chemistry. The end result is that the operating cost of labs can be substantially reduced.

Figure 1. MS/MS ion trace profiles (m/z 147 → 103) of coumarin spiked at 2 mg/kg (black traces) versus blank (red traces) in imitation vanilla extract (A), coffee (B), cookie (C), and ice cream (D).
GOAL
To successfully screen for the presence of di(2-ethylhexyl) phthalate (DEHP) in food and beverage products using a simple, rapid technique with minimal sample preparation and no chromatographic separation.

BACKGROUND
Di(2-ethylhexyl) phthalate (DEHP) is a cost-effective general purpose plasticizer used mainly for making plastics soft and pliable in building materials such as flooring, cables, as well as medical devices.

In May 2011, the Taiwan Food and Drug Administration (TFDA) found DEHP in powdered probiotics, which was then traced back to the clouding agent supplier. The so-called “clouding agent” is a legal food additive that is commonly used in beverage, food, and dietary supplements. However, the supplier intentionally replaced the additives with DEHP in order to cut costs. DEHP is 20 times more toxic than melamine, and is classified as a class B2, probable human carcinogen, under the U.S. Environmental Protection Agency. Consumption of plasticizer-tainted food or beverage products increases the risk of reproductive abnormalities.

Screen for DEHP in less than 2 minutes with minimal sample preparation and no chromatographic separation.

Figure 1. TIC traces of DEHP spiked at 1 mg/kg (blue) versus blank (red) in flavored syrup (A), fruit juice (B), fruit jam (C), and health supplement tablet (D).
The regulations of Food Containers and Appliances (Taiwan) mandate that the maximum level of DEHP dissolved from plastic items must not exceed 1.5 ppm, and that no DEHP can be added to food products. The international acceptance criteria of daily maximum consumption ranges from 1.2 to 8.4 mg for a 60 kg adult.

Food safety regulators require that all products found to be contaminated with DEHP be recalled and removed from the shelves immediately. Rigorous tests must be carried out on six categories of food and beverages including sports drinks, fruit juices, tea drinks, fruit jam and jellies, food powders, and health supplement tablets. This poses a major analytical challenge, as the complexity of food matrices requires the use of different extraction techniques. Thus the ability to rapidly screen for the presence of DEHP using a simple technique with minimal sample preparation and no chromatographic separation would be advantageous.

**THE SOLUTION**

The Waters® Atmospheric Solids Analysis Probe (ASAP), together with the Xevo® TQ MS System, is able to rapidly screen for the presence of DEHP in less than two minutes. Minimal sample preparation and no chromatographic separation are required for this analysis. This method is able to detect for DEHP confidently in a range of food matrices.

The glass capillary was dipped into the food sample and any excess sample was wiped off. The capillary was then attached onto the ASAP probe and loaded directly into the source enclosure of the Xevo TQ MS System. The desolvation gas was rapidly heated to 450 °C within 20 seconds and acquired using multiple reaction monitoring (MRM) with three transitions listed in Table 1.

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Table 1. MRM transitions for DEHP analysis.

**SUMMARY**

DEHP was successfully detected at 1 ppm in a range of food matrices. Requiring minimal sample preparation and with no chromatographic separation, this was achieved in less than 2 minutes per sample using ASAP and Xevo TQ MS.

This solution provides an increase in lab productivity and efficiency due to the ease of sample preparation (direct sampling from the matrix) and shorter analysis times. At the same time, with the minimal use of solvent in this analysis, the cost of lab consumables is also minimized.
AIM
To successfully screen for the presence of melamine in a range of milk-based food products using a simple, rapid technique with minimal sample preparation and no chromatographic separation.

BACKGROUND
Melamine is commercially used for whiteboards, floor tiles, kitchenware, fire retardant fabrics, and filters. In March 2007, melamine was found in wheat gluten and rice protein concentrates used in the manufacture of pet food. This caused the deaths of a large number of dogs and cats due to renal failure.

In September 2008, a second global scare arose involving milk, infant formula, and milk-based food products, adulterated with melamine. China reported an estimated 300,000 victims with six infant fatalities from kidney stones and other renal damage.

Why did this happen?
The procedure of adding melamine to animal feed has existed for many years so the practice is accepted. Melamine passes through routine protein tests undetected and artificially increases the Kjeldahl nitrogen test result for protein content. Ultimately, it is added to improve profits as there are low profit margins in bulk milk products. It is easy and cheap to obtain as there is little regulation for this compound.

In less than 2.5 minutes per sample, ASAP together with ACQUITY TQD can rapidly screen for the presence of melamine at levels relevant to legislation in a range of sample matrices.

Figure 1. Melamine (m/z 127 → 60) spiked at 2.5 mg/kg (purple traces) versus blank (green traces) in milk (A), infant formula (B), chocolate (C), and biscuit (D).
Worldwide response

Regions responded rapidly and differently to the levels that were thought to be safe and the products that were necessary to test.

- U.S. FDA and EFSA tolerable daily intakes were set at 0.63 and 0.5 mg/kg body weight, respectively
- International variation of limits
  - Hong Kong, food = 2.5 mg/kg, infant formula = 1 mg/kg
  - In Taiwan, melamine should not be detected in any food
  - EU, 2008/798/EC: foods containing >15% milk must be tested. Food containing melamine >2.5 mg/kg would be destroyed

The legislation mandates that the concentration of melamine in food products needs to be monitored so contaminated batches can be destroyed. This creates many analytical challenges when the food produce for testing is diverse, e.g., whole milk, infant formula, chocolate and biscuit. The ability to rapidly screen for the presence of melamine using a simple technique with minimal sample preparation and no chromatographic separation would be advantageous.

THE APPROACH

The Atmospheric Solids Analysis Probe (ASAP) together with ACQUITY® TQD is able to meet this demand. With minimal sample preparation and no chromatographic separation this solution is able to rapidly detect the presence of melamine at levels relevant to legislation in a range of sample matrices.

1 µL milk, infant formula, or the supernatant from chocolate and biscuit shaken with acetonitrile, were directly loaded onto the ASAP probe using a positive-displacement pipette. The ASAP probe was inserted into the sealed source enclosure and the desolvation gas was ballistically heated to 400 °C. The ACQUITY TQD was set up in multiple reaction monitoring (MRM) mode to acquire three transitions as listed in Table 1.

![The Atmospheric Solids Analysis Probe (ASAP).](image)

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<th>Precursor ion</th>
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<th>Cone voltage (V)</th>
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Table 1. MRM transitions for melamine.

SUMMARY

In less than 2.5 minutes per sample, ASAP together with ACQUITY TQD is able to rapidly screen for the presence of melamine at levels relevant to legislation in a range of sample matrices.

The detection of melamine was completed with minimal sample preparation and no chromatographic separation.

The benefits of this solution for a revenue conscious laboratory can be realized with increased efficiency through analytical time savings and decreased need for sample preparation, resulting in increased lab productivity. Cost savings can be made by lowering the use of lab consumables with the environmental impact of solvent usage also being reduced.
High Throughput Screening of Food Contact Materials

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APPLICATION BENEFITS
The use of the ASAP probe can substantially reduce the time of analysis, producing qualitative results and identification of potential migrants with increased confidence when used in conjunction with high resolution MS detection techniques, such as time-of-flight (ToF) MS. The use of ToF-MS also allows full scan screening of the samples so potential migrants other than those specifically analyzed for may also be detected.

INTRODUCTION
Most food and drink is packaged in some way. It is also highly likely that it comes into contact with other materials during harvesting, production, transport, storage, and cooking. A food contact material (FCM) is any material or article intended to be placed in contact with foodstuffs! Food packaging materials are the most notable example, but also included are cutlery, dishes and plates, containers, parts of food processing equipment, etc.

When food comes into contact with a FCM there is the potential for migration of any of the chemicals from the material into the foodstuff. Depending on the chemical substance(s) involved, this can compromise the safety and/or the quality of the food, and so most countries have legislation in place to keep any chemical migration within acceptable limits. In Europe the EU Framework Regulation (EC) No. 1935/2004 provides general requirements for FCMs. Article 3 states that they should not endanger human health, bring about an unacceptable change in composition, or deteriorate any organoleptic characteristics.

Further to this framework regulation is more specific legislation. One example is the migration of primary aromatic amines (PAAs) which are regulated through the Plastics Directive 2002/72/EC, as amended, which states that:

- Plastic materials and articles shall not release primary aromatic amines in a detectable quantity (DL = 0.01 mg/kg of food or food simulant). The migration of the primary aromatic amines appearing in the lists in Annex II and III is excluded from this restriction.

Over the last couple of years there have been numerous notifications relating to the migration of PAAs from nylon kitchen utensils via the Rapid Alert System for Food and Feed (RASFF). As concerns to human health grow regarding these FCMs, quicker and easier methods need to be developed to screen for compounds in the current legislation. This application note will detail the analysis of nylon kitchen utensils for PAAs and will show how the latest advances in mass spectrometer probe design help to achieve this goal.
Variables such as cone voltage, desolvation gas (nitrogen) temperature and corona pin current were optimized using solvent standards. Once the optimum settings were achieved the screening of the sample took a matter of minutes. The ASAP probe was used in the usual way; a new glass capillary was used for each sample removing sample carryover giving results that were more reliable by minimizing false positives.

The glass capillary was inserted into the source chamber at an elevated temperature for approximately one minute. This cleaned any contamination from the tip. The probe was then removed, cooled and the glass tip wiped backwards and forwards across the surface for 10 seconds. The mass spectrometer was set to an optimum desolvation gas temperature and the probe reinserted into the Xevo G2 QToF and the signal created recorded. This manual screening process was performed as quickly as 3 minutes per sample.

RESULTS AND DISCUSSION

Keeping a check on the migration of all the starting substances that may be used to make FCMs is a massive undertaking. This involves the chemical analysis of either the material itself or testing for migration of chemicals into foods or into model foods that are called food simulants. For this mass spectrometric methods and especially gas chromatography with mass spectrometric detection (GC-MS) and liquid chromatography with mass spectrometric detection (LC-MS) are widely used.

The use of the ASAP probe can substantially reduce the time of analysis, producing qualitative results and identification of potential migrants with increased confidence when used in conjunction with high resolution MS detection techniques, such as time-of-flight (ToF) MS. The use of ToF-MS also allows full scan screening of the samples so potential migrants other than those specifically analyzed for may also be detected.
Two different sampling techniques were tested to see which would achieve the better results. The ASAP probe was wiped across the surface of the kitchen utensils and then inserted into the MS. A fine powder was also prepared from the sample using sandpaper and the probe rubbed in this powder before insertion into the MS. The strongest signal was seen for the powder approach, and the results for the two samples are shown in Figure 2.

Sample A was found to contain levels of aniline and 4,4'-MDA ([M+H]+ adduct seen in both cases). PAAs were not detected in sample B. The total ion chromatogram gives the location of the peak on the trace, showing that the compounds are not present. These were the only compounds to give a positive result for these samples.
A high degree of confidence was achieved with the identification of these compounds. All of the spectra across
the 4,4'-MDA peak were assessed with respect to mass accuracy of the system. Figure 3 shows the spectrum
acquired at the apex of the peak (spectrum 11), the total mass accuracy across the peak is shown in Table 1.

Having identified sample A as a potential positive, it clearly merits being subjected to migration testing using
food simulants to see if it complies or not with migration limits for the PAAs identified.

![Figure 3. Spectra of 4,4'-metricalinedianiline, m/z 199.1235.](image)

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Table 1. The mean mass accuracy of the 22 data points is 0.7 ppm for the 4,4'-MDA [M+H]+ ion, m/z 199.1235.
This data was acquired using a Xevo G2 QToF in ToF mode. Further analysis of the data after it has been acquired is possible. In this example, the aim of the experiment was to look for PAAs, but examination of the ToF data revealed other potential migrants that were identified. Post acquisition interrogation of this sort would not be possible if a quadrupole MS system was used for the analysis that only acquired the data in SIR or MRM modes.

![Figure 4](image)

**Figure 4.** Further analysis of Sample A reveals that Di-n-butyl phthalate (DBP), Di-(2-ethylhexyl) phthalate (DEHP), Di-n-octylphthalate (DnOP), and/or Di-isodecyl phthalate (DIDP) are also present. The mass accuracy of the Xevo G2 QToF does not show any error, even when many compounds are being ionized at the same time.

The presence of some common phthalates in sample A is shown in Figure 4. A chromatographic separation is needed to allow quantification of the isobaric DEHP and DnOP. As phthalates are ubiquitous in the environment the presence of phthalates may be due to contamination of the nylon sample. Further abrasion and testing would prove the origin.
CONCLUSIONS

■ Using the Xevo G2 QTof, in ToF mode, with an ASAP probe is a fast and easy method to screen for potential migrants from food contact materials.

■ Sample preparation times for this approach can be less than 3 min per sample, allowing increased throughput and revenues to be maximized.

■ Xevo G2 QTof allows for interrogation of data for compounds that were not on the original screening list when the analysis occurred.

■ Xevo G2 QTof raises the level of confidence in results with excellent mass accuracy.

References
