ION MOBILITY-MASS SPECTROMETRY: A NOVEL APPROACH TO SCREENING FOR EXTRACTABLE AND LEACHABLE COMPONENTS FROM PACKAGING MATERIAL

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INTRODUCTION
Extractable and leachable components, which are potentially harmful to human health, are of great concern to manufacturing industries, particularly manufacturers of food contact materials (FCM), and materials intended for use in the pharmaceutical industry. Globally, much legislation exists¹⁻⁷ to try to mitigate exposure to these components, which results in a significant demand for rapid, accurate, and reliable analytical methodologies. One such method is targeted screening using LC-MS techniques. Typically, in identification of compounds, retention time, accurate mass, and fragmentation ion information is used. However, if different chromatographic methods are used, the retention times will vary. In this work, we demonstrate how the inclusion of collision cross section (CCS) values, acquired using ion mobility-mass spectrometry, can provide increased confidence in compound identification.

Ion mobility technology, such as Vion IMS QTof or SYNAPT G2-Si HDMS, enables the determination of collision cross section (CCS), which is a key physicochemical property of compounds. The CCS depends on an ion’s size, shape and charge. For example, in the case of two ions with the same m/z but different shapes, the less compact, straight-chain species will have a longer drift time than the smaller, more compact species (Figure 1). The ion mobility specific part of novel UNIFI software was used to identify components based on their exact mass and CCS, thus reducing the reliance on reproducible chromatographic retention times.

METHODS

UPLC conditions:
Column 1: ACQUITY UPLC BEH Phenyl Column, 130 Å, 1.7 μm, 2.1 mm X 100 mm
Column 2: ACQUITY UPLC CSH C18 Column, 130 Å, 1.7 μm, 2.1 mm X 100 mm
Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in methanol
Flow rate: 0.4 mL/min
Gradient: see Table 1
Column Temperature: 40 °C
Injection volume: 10 μL

MS conditions:
Ionization mode : ESI pos
Capillary voltage: 3.0 kV
Cone voltage: 30 V
Desolvation temperature: 450 °C
Desolvation gas flow: 800 L/Hr
Cone gas flow: 10 L/Hr
IMS Wave velocity: 550 m/s
IMS Wave height: 40 V
IMS cell pressure: 3.5 mbar

Six different dye compounds were chosen to illustrate the methodology. Each dye was prepared in methanol at 100 ppb level and their CCS values were determined. Subsequently, one isomer of each dye was spiked in plastic packaging extracts in isopropanol at 100 ppb level and analyzed for targeted screening experiments.

Data Acquisition and Processing
Data were acquired using MassLynx v4.1 and processed using UNIFI v1.8 software.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>%A</th>
<th>%B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.40</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>5.00</td>
<td>0.40</td>
<td>0</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>9.00</td>
<td>0.40</td>
<td>0</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>9.10</td>
<td>0.40</td>
<td>90</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>10.00</td>
<td>0.40</td>
<td>90</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1. LC-MS gradient
RESULTS AND DISCUSSION

Three isomeric pairs of dyes: 2,4-diaminotoluene (2,4-DAT), 2,6-diaminotoluene (2,6-DAT), 3-chloro-4-methoxyaniline (3-C-4-MA), 5-chloro-2-methoxyaniline (5-C-2-MA), 2,4-dimethylaniline (2,4-DMA) and 2,6-dimethylaniline (2,6-DMA) were selected for this experiment (Figure 2). These types of compounds are used as dyes, or in the manufacturing of pigments. A summary of CCS values and retention times for individual standards are shown in Table 2.

If the retention time is not known and limited fragmentation information is observed (at trace levels, the fragments might be absent due to low levels of precursor available for fragmentation), it is very difficult to distinguish which isomer is which based on just accurate mass information.

In Figure 3, the results are summarized for a standard of 5-chloro-2-methoxyaniline. The software lists 2 choices for the detected accurate mass. However, the expected CCS value for the other isomer 3-chloro-4-methoxyaniline is more than 6% different than the expected value for the 5-chloro-2-methoxyaniline. The limits have been specified in the processing method to give a warning if any CCS is more than 2% different from the expected value, and flag as an erroneous assignment if CCS is more than 5% different from the expected value (indicated by the red colored cell in the table in Figure 3). Typically, the observed CCS values using ion mobility systems like Vion or SYNAPT are within 2% from the expected value. The results display also the extracted ion chromatogram for the compound, as well as low and high energy spectra for the compound. The fragmentation information from the high collision energy data can further assist in confirmation of the correct assignment for the m/z of interest.

Similar results were observed for the other 2 pairs of isomers. In Figure 4, the isomer of 2,6-diaminotoluene has an observed CCS value which is greater than 2% but less than 5% different from its expected CCS. In this case, the software flags it with a yellow warning, to draw the analyst’s attention to the result. For 2,6-dimethylaniline, its isomer has an expected CCS value which is more than 6% different from the expected CCS. In each case, the 2,4- isomers were correctly identified, and the addition of the CCS value has helped to make a confident assignment when retention time information is not available.

Table 2. CCS values and retention times for the dye standards using BEH Phenyl column

<table>
<thead>
<tr>
<th></th>
<th>2,4-DAT</th>
<th>2,6-DAT</th>
<th>3-C-4-MA</th>
<th>5-C-2-MA</th>
<th>2,4-DMA</th>
<th>2,6-DMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCS (Å²)</td>
<td>61.98</td>
<td>64.28</td>
<td>71.27</td>
<td>66.73</td>
<td>64.69</td>
<td>61.96</td>
</tr>
<tr>
<td>RT (min)</td>
<td>1.10</td>
<td>0.88</td>
<td>2.19</td>
<td>3.27</td>
<td>2.36</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Figure 2. Structures for the dyes
Ion Mobility-Mass Spectrometry: A Novel Approach to Screening for Extractable and Leachable Components from Packaging Material

Table 3. CCS values and retention times for the dye standards using the CSH C18 column

<table>
<thead>
<tr>
<th>Component name</th>
<th>CCS (Å²)</th>
<th>RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-DAT</td>
<td>61.80</td>
<td>0.53</td>
</tr>
<tr>
<td>2,6-DAT</td>
<td>63.75</td>
<td>0.50</td>
</tr>
<tr>
<td>2-C-MA</td>
<td>70.99</td>
<td>0.62</td>
</tr>
<tr>
<td>3-C-MA</td>
<td>66.95</td>
<td>3.17</td>
</tr>
<tr>
<td>2,4-DMA</td>
<td>64.16</td>
<td>0.62</td>
</tr>
<tr>
<td>2,6-DMA</td>
<td>61.57</td>
<td>2.18</td>
</tr>
</tbody>
</table>

The accuracy of identification was tested by using an IPA extract of a generic pill bottle. One isomer of each dye was spiked at 100 ppb level. As an example, 2,4-dimethylaniline was correctly identified (2,6-dimethylaniline was not spiked into the extract). This result is shown in Figure 5. Since the extract is in IPA, and the strong solvent in the mobile phase is methanol, a split peak was observed. In either case, the correct identification of the isomer is made based on CCS value (within 1.52%).
CONCLUSION

- Ion mobility technology provides information on the size and shape of the compounds by using the drift time to calculate CCS.
- CCS is an additional parameter for confident identification in targeted screening for isomers in the absence of retention time.
- In certain cases, when using different chromatographic methods for screening, CCS can account for the resulting differences in retention times, thus enabling the use of CCS libraries created using different acquisition methods.

References