1. Introduction

Glyphosate is currently one of the most common pesticides used worldwide. In spite of its approval by regulatory bodies all over the world, the concern about its harm to humans and the environment persists. Therefore, the strict control of Glyphosate and its metabolite Ammoniumglycylphosphonic acid (AMPA) in food and environment is mandatory. The chromatography of glyphosate is challenging due to its high polarity. In order to overcome this, there exists a well-established method including a derivatization step with 9-fluorenlylmethoxychloroformate (FMOC) followed by LC-MS analysis. Here we report a fully automated derivatization followed by LC-MS/MS analysis of beer samples. The instrumental set-up does not require any additional hardware for sample pretreatment but uses the built-in pretreatment function of the autosampler.

2. Methods and Materials

2.1 Sample Preparation

After precipitation with methanol (50:50) and centrifugation the beer samples were set into the autosampler.

2.2 UHPLC method

Instrument: Nexera UHPLC, Shimadzu
Column: Genome 5 µm C18, 150 x 2.1 mm
Mobile phase: A: 2 mm NaH₂PO₄, pH 3.5
B: acetonitrile
Flow rate: 0.4 mL/min
Time program: B conc. 5%(0 min) - 50%(7 min) - 95%(7.01-12 min)
Injection vol.: 50 µL
Column temperature: 35 °C
Injection: pumping ESI
Nebulizing gas: 3 L/min
Heating gas: 3 L/min
Drying gas: 5 L/min
Interface temperature: 325 °C
Heat block temperature: 400 °C
CID gas: 270 kPa
Interface voltage: 4 kV/ -3 kV
Heating gas: 15 L/min
Stop reaction: done by SIL-30AC within 15 minutes
LCMS conditions

<table>
<thead>
<tr>
<th>Method</th>
<th>Component</th>
<th>Conc.</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glyphosate</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>B</td>
<td>Glyphosate</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>C</td>
<td>Glyphosate</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
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<tr>
<td>D</td>
<td>Glyphosate</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>E</td>
<td>Glyphosate</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
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</tbody>
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3. Results

3.1 Method development for automatization of derivatization

The addition of internal standards as well as the derivatization of Glyphosate and AMPA with FMOC was done fully automated by the autosampler SIL-30AC within 15 minutes. After derivatization the sample was injected directly to the LC-MS/MS and analysed accordingly.

3.2 Quantitative Analysis of 40 beer samples

A total of 40 commercially available beer samples were analysed. Among these samples there were 21 samples of beer brewed according to Pilsener style, 3 samples of organic beer, 10 samples of other types of beer and 6 samples of alcohol-free beers or non-alcoholic beer mix drinks. All samples were analyzed in duplicate in two consecutive runs. While Glyphosate was detected in 85 % of all samples its metabolite AMPA was below LOQ in all samples.

4. Conclusions

- Fully automated FMOC-derivatization of Glyphosate and AMPA within 15 minutes.
- No additional hardware required
- Sample derivatization and internal standard addition done by autosampler SIL-30AC
- Maximized sample throughput due to overlapping sample pretreatment functionality
- Robust and reliable method for Glyphosate and AMPA even in a complex matrix like beer.

The products and applications in this presentation are intended for Research (RUO). Not for use in diagnostic procedures.