Objective

Our research was aimed at establishing and validating a quick analysis method for determining selected polyphenols in cocoa and chocolate products using HPLC-FD. Quantification of the flavonoids epicatechin, catechin, procyanidin B2, and procyanidin C1 in the samples was performed using external standards.

Sample Processing

Weighing in of approx. 1 g of sample into centrifuge tube

Defatting using 3 x 10 ml hexane

Extraction using 2 x 5 ml MeOH:H2O (80:20, v/v)

SPE cleanup

HPLC Parameters

- Column: ultraPFPPP (100 x 2.0 mm, 3 µm)
- Oven temperature: 30°C
- Injection volume: 1 µl
- Eluent: A: 0.1% formic acid
  B: 0.1% formic acid in acetonitrile
- Fluorescence detection: excitation at 280 nm, emission at 318 nm
- Mode: gradient (cf. Figure 2)

Figures 3 and 4 show the HPLC-FD chromatogram of the mixed standard containing the flavonoids (+)-catechin, (+)-epicatechin, procyanidin B2 and C1 and a chocolate sample respectively.

Validation

The presented method was validated based on the guidelines provided by Kromidas [4]. For this purpose a chocolate sample was prepared and analysed according to the method developed within the scope of this study. Table 1 shows a summary of the key validation results, such as the relative standard deviation of repeatability (RSDr) and reproducibility (RSDs), the recovery rate, and the limit of detection (LOD) and limit of quantification (LOQ) of the four examined flavonoids.

<table>
<thead>
<tr>
<th>Table 1: method validation results</th>
<th>Catechin</th>
<th>Epicatechin</th>
<th>Procyanidin B2</th>
<th>Procyanidin C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability RSDr [%]</td>
<td>5.1</td>
<td>4.6</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Reproducibility RSDs [%]</td>
<td>6.1</td>
<td>5.3</td>
<td>5.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Recovery rate [%]</td>
<td>75.5</td>
<td>81.4</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>LOD (µg/g)</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>LOQ (µg/g)</td>
<td>15</td>
<td>27</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>(n.d. = not determined)</td>
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</table>

Conclusion

A quick and simple analytical method for determining selected polyphenols (catechin, epicatechin, procyanidin B2 and procyanidin C1) in cocoa and chocolate products has been successfully established and validated. This method is a suitable procedure for routine analysis. After only a little preparation, the sample extract can be analysed within a short time using HPLC-FD and the flavonoids contained in the sample can be qualitatively and quantitatively determined via external standards.

References