Abstract

On-line coupling of an automated flow system with a commercially available capillary electrophoresis (CE) system with an electrospray interface (ESI) for mass spectrometric (MS) detection is described.

The peculiarities of CE–ESI–MS interfaces, in which a high electrical field must be applied to the capillary end where the sample is provided by the flow system, introduce significant difficulties for the appropriate work of the entire arrangement. Experimental strategies are proposed for achieving stable conditions for on-line sample pre-treatment, conditioning of the separation capillary, sample injection, and the proper separation. The versatility and robustness of the proposed arrangement is discussed, taken as an example the separation of a variety of amines. Connection of the CE systems pressure to the automated flow system enables hydrodynamic introduction of sample with high precision.

The developed hyphenated system is of practical relevance as it opens an avenue for the simplification and automation of the whole analytical process required when using powerful CE–ESI–MS equipments.

Experimental Set-up

For this configuration the amount of BGE located between the water and the interface must be enough for a complete CE separation. Considering the capillary volume of the interface, a constant flow of the BGE must be maintained to avoid possible changes in its composition.

It means that long separation times require long coils. The BGE must be introduced into the coil 2 in several stages to avoid that the BGE reaches the automatic burner due to dispersions in the coil.

The length of the reaction coil is determined by the following criteria:

(i) Separation time: The regeneration of the BGE in the interface flow required during the electrophoretic separation is based on the fact that the sample must reach the interface and the valve of the flow system in the reaction coil. Typically we use a coil of 300 cm for separation times of 30 min. To facilitate introduction of a new buffer and to reduce the analysis time, it is interesting to use the same BGE electrolyte as the carrier in the flow system. In this case, the cleaning and conditioning of the reaction coil will be faster.

The automated flow system was coupled to a CE–ESI–MS equipment using a capillary/micro polymer emitter column (MS). The interface was set under the conditions described by the supplier. It means that the interface can slightly be modified because of the instrument needs to detect a vial in the inlet position and then start the separation and the conditioning of the flow system.

The CE–MS coupling is done by means of a T-piece coupling of an automated flow system with a commercially available MS equipment and an interface of capillary electrophoresis, in which a high electrical field must be applied to the capillary end where the sample is provided by the flow system.

Use of deionized water plugs for avoiding dissipative currents

One approach to avoid dissipative currents consists in the introduction of deionized water between the interface and the automated flow system.

In this case, the cleaning and conditioning of the reaction coil will be faster.

Use of air plugs for avoiding dissipative currents

An interesting alternative to isolate the electrode from the safety line is insertion of an air plug between the interface and the valve of the flow system. In this case, the cleaning and conditioning of the reaction coil will be faster.

Analysis of synthetic samples

The reliability of the method was evaluated by the analysis of several synthetic water samples. The recoveries of target analytes ranged from 80% to 105%.

Sample Amount Amount Recovery

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Amount (mg.L⁻¹)</th>
<th>Amount (mg.L⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Histidine</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Uncinate</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Hydroxyphenylalanine</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

The statistical analysis of the calibration curve demonstrates the high efficiency of the proposed arrangement for coupling automatic flow systems to CE–MS equipment.

Conclusions

The robust on-line coupling of an automated flow system with a CE–MS system introduces a new powerful integration of different analytical techniques.

Sample pre-treatment, including reaction and sample clean-up steps, can be performed in a automated fashion. The treated sample can then be separated in its components taking advantage of the high resolving power of modern CE. Finally, important advantages in the detection stage in terms of selectivity and sensitivity can be achieved by MS. Therefore, it may be expected that this hyphenation can be applied for solving the spectrum of a broad variety of different analytical problems.