"The Theory of Everything" meets "The Imitation Game": Quantification of amino acids, potential biomarkers of ALS, by CE-C4D with the usage of Excel VBA programming for faster data processing

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Abstract
Amyotrophic lateral sclerosis (ALS) is a rare disease with unknown cause and no treatment or fast tests for diagnosis. Some amino acids are potential biomarkers of ALS, which could aid the disease diagnosis. In this work, capillary electrophoresis with C4D detection was used to quantify such analytes in blood plasma within a 10 min run using 3 mol/L acetic acid + 0.1% hydroxyethyl cellulose (HEC) as BGE. An Excel VBA program was developed and minimized 50 fold the time spent on statistical validation of the analytical curves.

Key words: Capillary electrophoresis, ALS, Excel VBA

Introduction
Although rare, ALS is a devastating disease with a 2 to 5 years life expectancy. The aim of this study is to devise means to determine whether glutamic acid (Glu), cysteine (Cys), homocysteine (Hcy) and methionine (Met) may be characterized as ALS biomarkers, thus helping the prognosis, diagnosis and treatment of the disease.

The construction of each analytical curve for analytes quantification demands a series of statistical validations that takes more than 10 minutes per attempt. Using a program to do such repetitive work diminishes drastically the data processing time.

Results and Discussion

The separation of the four amino acids and an internal standard (NL) by CE-C4D takes 10 minutes and was achieved by diluting the sample in acetonitrile:water 1:1 (v/v), using 3 mol/L acetic acid with 0.1% HEC as background electrolyte (BGE) (Image 1). The method was validated with a pool of plasma from healthy subjects, using 200 µL per analysis. Quantification limits (LQ) were determined by signal/noise ratio = 10.

Table 1. Figures of merit of the method.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Hcy</th>
<th>Met</th>
<th>Glu</th>
<th>Cys</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD*</td>
<td>4,220</td>
<td>2,169</td>
<td>6,518</td>
<td>24,48</td>
</tr>
<tr>
<td>Linear range*</td>
<td>5-50</td>
<td>20-100</td>
<td>20-250</td>
<td>50-250</td>
</tr>
<tr>
<td>r</td>
<td>0.9981</td>
<td>0.9998</td>
<td>0.9984</td>
<td>0.9986</td>
</tr>
<tr>
<td>Precision</td>
<td>6.31%</td>
<td>2.05%</td>
<td>6.20%</td>
<td>2.32%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>2.90%</td>
<td>1.71%</td>
<td>8.34%</td>
<td>1.00%</td>
</tr>
</tbody>
</table>

*concentrations in µmol/L.

The obtained lineairities were better than 0.98, and precision and accuracy showed CVs smaller than 10%, showing that the method is valid for the evaluated analytes.

Conclusions
The analytical curves for all the studied amino acids were successfully validated, showing lineairities above 0.9980 and precisions and accuracies below 10%. The developed VBA program dropped data processing time from 10 min to 15 sec. The analysis of ALS patients’ samples is underway.

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