"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
diagnosis1. 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.

![Image 1](https://example.com/image1.png)

**Image 1.** a) Electropherograms and b) analytical
curve for methionine.

To calculate the method figures of merit (Table 1),
a program in VBA language was built from an
Excel® workbook used in previous works. Such
workbook was designed to calculate a linear
regression upon the entrance of the experimental
data and contained formulas to manually perform
statistical tests (outliers exclusion by Jackknife, F-
test and homoscedasticity test).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Hcy</th>
<th>Met</th>
<th>Glu</th>
<th>Cys</th>
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</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 linearity was better than 0.98, and
precision and accuracy showed CVs smaller than
10%, showing that the method is valid for the
evaluated analytes2.

Conclusions

The analytical curves for all the studied amino
acids were successfully validated, showing
linearities 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.

Acknowledgement