LT004

GL Sciences Inc.

# Simultaneous Analysis of Gamma Aminobutyric Acid (GABA) and 17 Amino Acid Components

Gamma-aminobutyric acid (GABA) is considered to be an effective treatment for strokes, heart disease, diabetes, menopausal disorders, and dementia, therefore it is an important compound that needs to be analyzed. Because of its structural properties, GABA can be analyzed with highly sensitivity and selectively using HPLC with fluorescence detection by derivatization with orthophthalaldehyde (OPA, which binds specifically to amino groups). This application note details a method for simultaneous analysis of GABA and 17 different amino acids.

| Between the analyses B | |
|---|---|
| Analysis time | 42 min |
| Analysis time, including column Equilibration | 80 min |

— Characteristics of the analytical procedure -

**1. Analysis can be made with high sensitivity**

→ OPA derivitization is about 70 times more sensitive than common analytical methods for amino acids (ninhydrin method, Standard Methods of Analysis for Hygienic Chemistry )

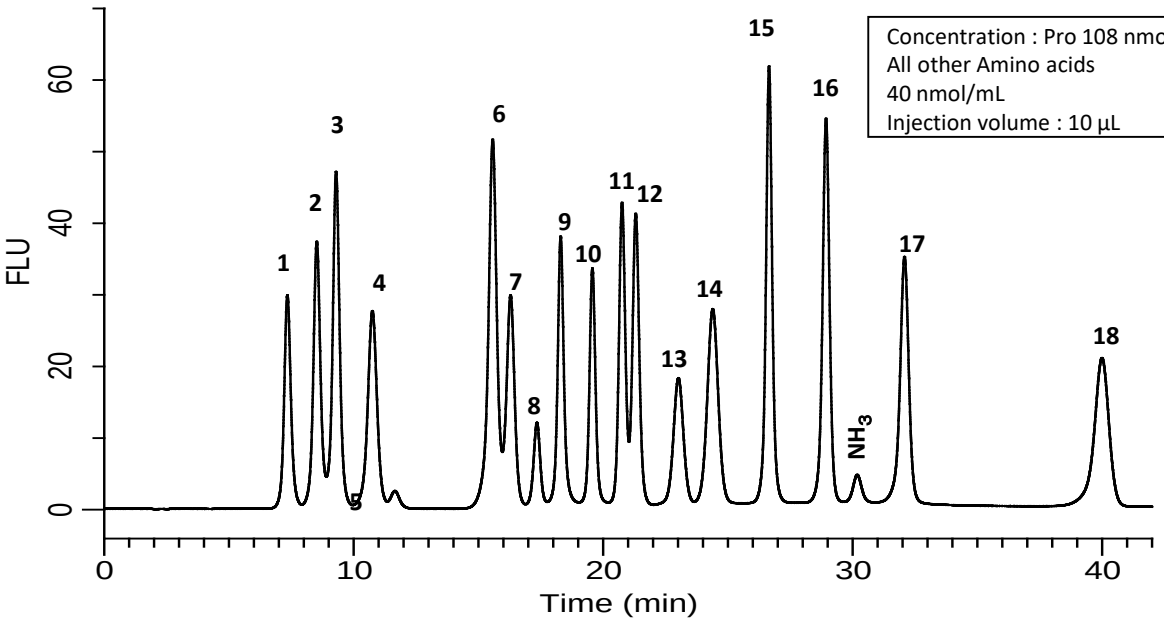
**2. Good reproducibility and quantitation**

→ The reaction in the post-column method is less susceptible to contaminants. For this reason, in the derivatization method. This system offers greater reproducibility and quantification, enabling high-precision analysis.

**3. Highly selective**

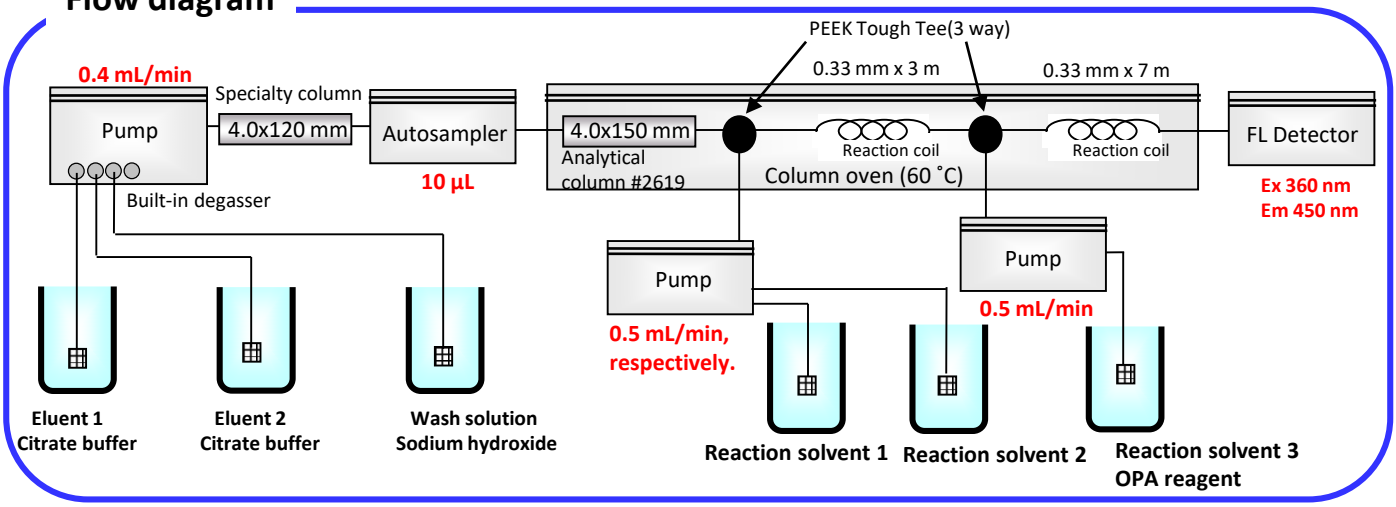
→ The fluorescent derivatization reagent with high reactivity and selectivity allows simple pretreatment for analysis without interference from contaminants.

1. Aspartic acid (Asp) 4. Glutamic acid (Glu) 7. Alanine (Ala) 10. Methionine (Met) 13. Tyrosine (Tyr) 16. Lysine (Lys)
2. Threonine (Thr) 5. Proline (Pro) 8. Cystine (Cys) 11. Isoleucine (Ile) 14. Phenylalanine (Phe) 17. Histidine (His)
3. Serine (Ser) 6. Glycine (Gly) 9. Valine (Val) 12. Leucine (Leu) 15. GABA 18. Arginine (Ala)




## Flow diagram

Examples of Analytical Samples of GABA and 17 Amino Acid Components (NH 3 is not analyzed.)

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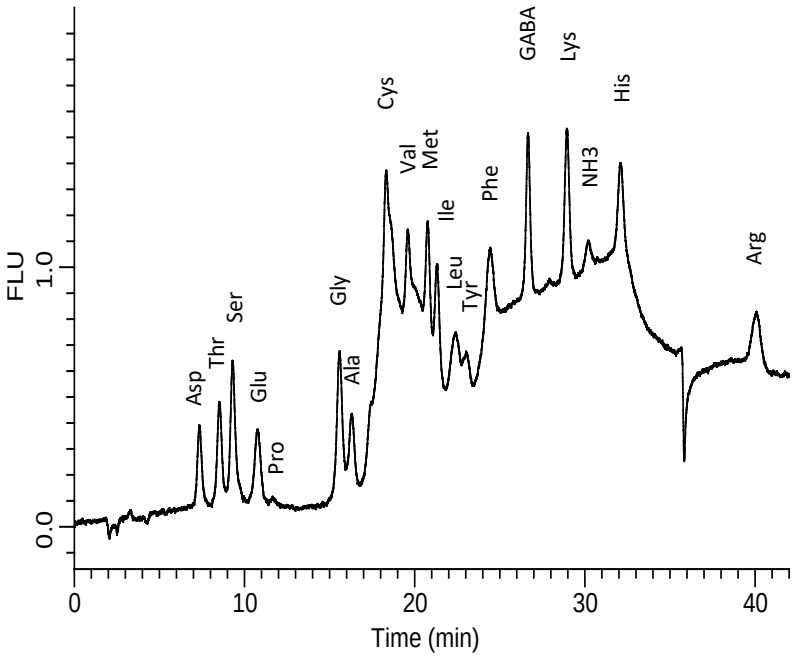
# Application

### Reproducibility and lower limitation of quantitation.

The CV values (*n*=3) for the retention times and areas in the chromatograms on the previous page showed good values within 0.5 % and 1.5 %, respectively.

The figure on the right shows an example of an assay of a standard sample (10 µL injection volume) prepared at a concentration of 1.08 nmol/mL for Pro and all other components at a 0.4 nmol/mL. These concentrations are required for quantitation using this method.

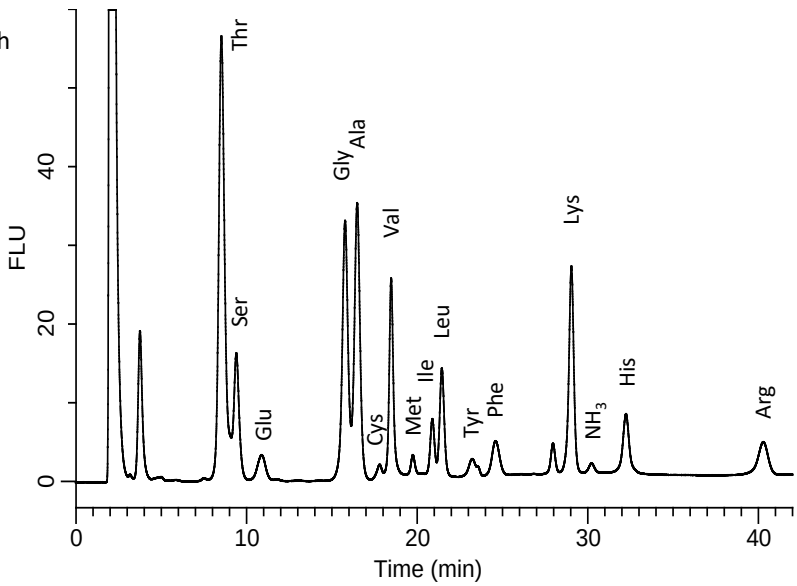



### Free amino acid analysis in human serum

The figure on the right is an example of free amino acid analysis in human serum. Serum was deproteinized with acidic solvent, centrifuged, and analyzed using supernatant. In this method, GABA was not detected in human serum.

**Pretreatment**

| | |
|---|---|
| Serum | 1 mL |
| Deproteinization | 5% Sulfosalicylic acid 9 mL |
| Stirring | |
| Centrifuge | 3000 rpm, 5 min, 4 ˚C |
| HPLC | Supernatant was injected with 10 µL |

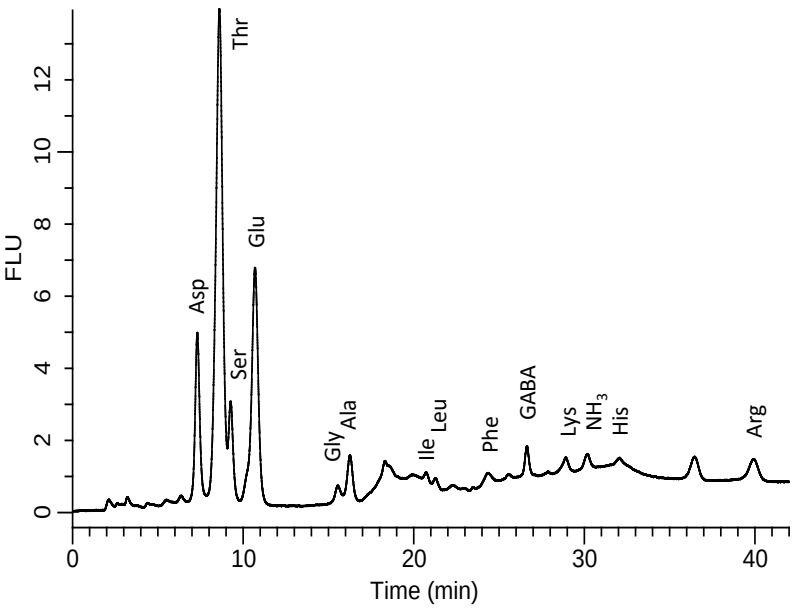



### Amino acid analysis in green tea

The right figure is an example of amino acid analysis in green tea. Green tea was diluted 10 times with an acidic solvent, filtered through a filter, and analyzed.

**Pretreatment**

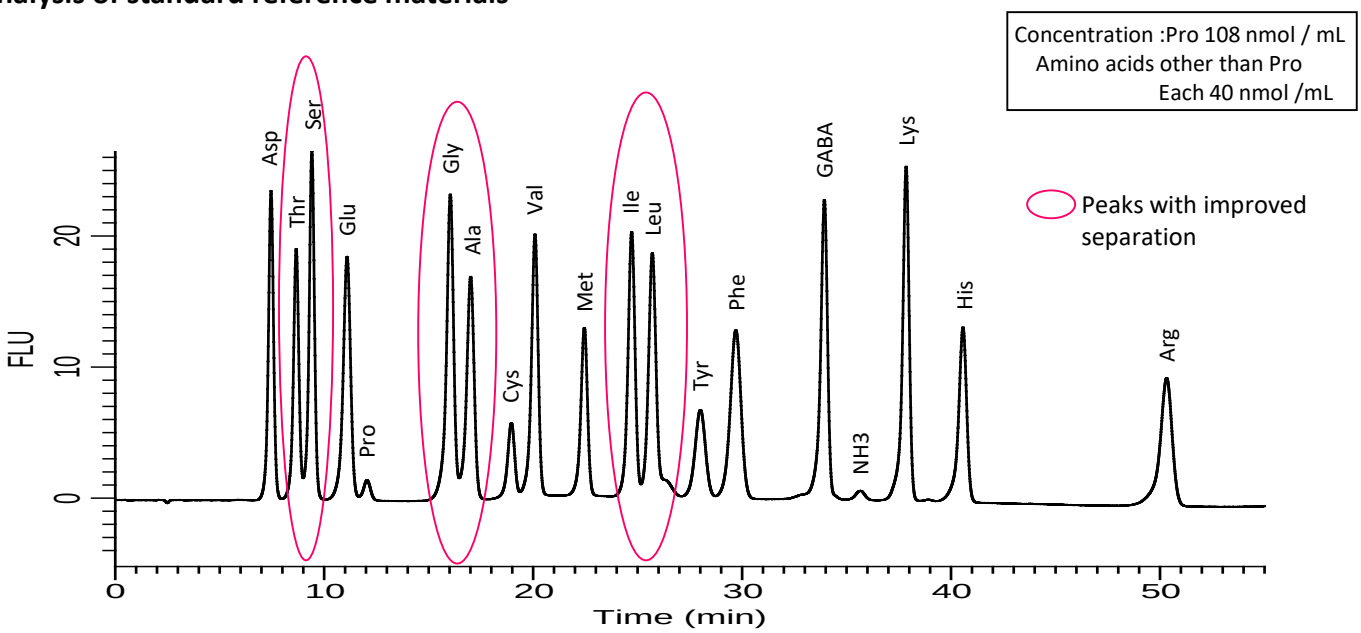
| | |
|---|---|
| Green tea | 1 mL |
| Dilute ⩾ | 0.1N HCl 9 mL |
| Filter N | Filter (GL Chromato Disk, pore size 0.45 µm |
| HPLC | Injecting 10 µL |



# Improved separation

The figure below shows an example of an analysis with improved separation of reference materials. The gradient elution was varied to improve the relative separation of Thr and Ser, Gly and Ala, and Ile and Leu. The assay time is 52 minutes (the assay time, including column equilibration, is 90 minutes).

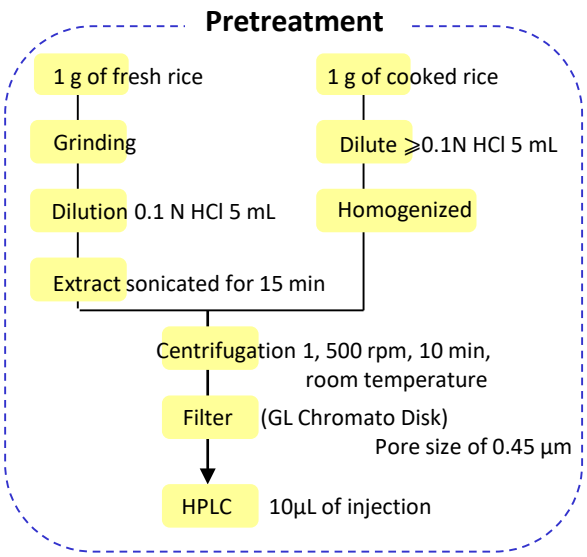
## Analysis of standard reference materials

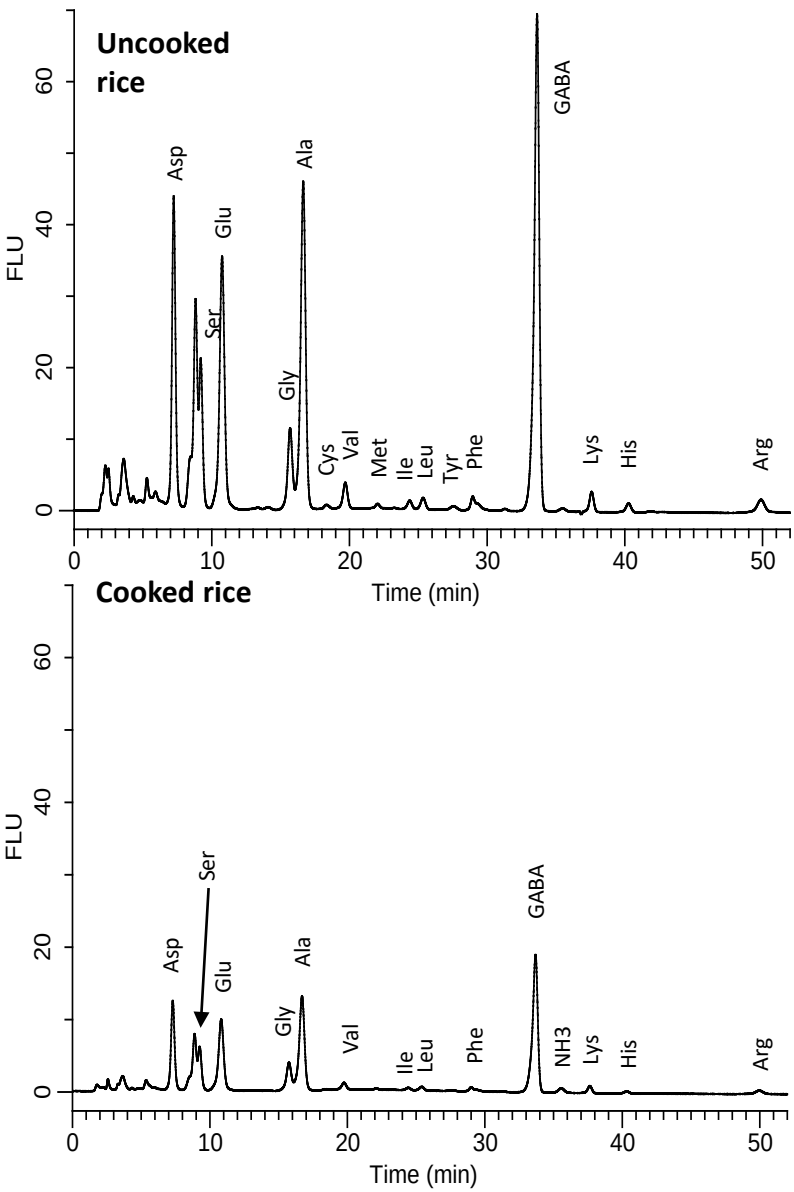


# Amino acids analysis in rice

Amino acid analysis in uncooked rice, cooked rice was performed. Uncooked rice sonicated while rice cooked was homogenized, and then each was centrifuged, and the supernatant was filtered through a filter and analyzed.

In 100 g of raw rice, 6.4 mg of GABA was found while 1.8 mg of GABA in 100 g of cooked rice was found.



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