Amino Acid Analysis of Spinach and Apple using a QuEChERS Sample Preparation Technique and Automated OPA/FMOC Derivatization LC Method

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Introduction

An automated online OPA /FMOC derivatization method for amino acids will be used to analyze QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extracts of apple and spinach. Amino acid analysis of the food extracts will be compared. Scalability, batch-to-batch reproducibility, linearity, and long-term stability of the derivatization method will be presented. Several column options will be shown, ranging from rapid nine minute analyses of 23 amino acids including re-equilibration using short (8µm) Rapid Resolution High Throughput columns (1.8mm) to 40 minute analyses using 250µm traditional 5 columns.

The LC Method

The Online Pre-Column Derivatizations

The primary amino groups react with fluorenylmethyl chloroformate (FMOC) at about pH 10 to form a stable derivative. Secondary amino groups do not react. The FMOC derivatized amino acid is then detected by fluorescence at about 330 nm.

The Automated Derivatization

G1310C plate automated liquid sampler (RLPLS):
1) Draw 2.5µL from Sample vial (Agilent PN 9851-3230)
2) Draw 19.5µL from Sample vial
3) Mix 16.5µL in washport SX
4) Wait 0.2 min
5) Mix 4.0µL in washport 10X max speed
6) Mix 4.4µL in washport 10X max speed
7) Draw 20µL from Injection Vial
8) Mix 19µL in washport SX
9) Mix 1µL in washport SX
10) Wait 0.5 min
11) Valve bypass

The Mobile and Stationary Phase

Stationary Phase: ZORBAX Eclipse Plus C18 Column Temperature: 40°C Mobile Phase A: 10 µL Na2B4O7, 10 µL NaH2PO4, pH 2.5
Mobile Phase B: Acetonitrile: Methanol: Water (40:60:50 v:v:v) Injection Diluent: (0.25 mL, H2O/MeOH = 100 mL)

The Linear Gradients

The gradients are specified for the C18 column. The different gradient delay times are calculated by reducing delay volume and the inertsin fluid fast at the beginning of the gradients program.

An Eclipse Plus C18 5µm Option

4.6 x 150 mm, 5 µm (pre-column)

A Rapid Resolution 3.5µm Option

4.6 x 150 mm, 3.5 µm

RRHT 1.8µm Options

4.6 x 250 mm, 1.8 µm (pre-column)

Amino Acid Identification and Detection

1. Alanine acid
2. Glutamic acid
3. Aspartic acid
4. Leucine
5. Valine
6. Isoleucine
7. Glycine
8. Threonine
9. Phenylalanine
10. Tyrosine
11. Threonine
12. Methionine
13. Histidine
14. Proline
15. Arginine
16. Lysine

Primary Amino Acids 1.20 µmol detected at 205nm wavelength 3200x
Secondary Amino Acids 0.10 µmol detected at 220nm wavelength 3200x

Flow chart of the Agilent SampleJet QuEChERS ADAC extraction procedure for pesticides in the grey boxes.

The QuEChERS Technique

Results

Spinach Leaf Amino Acids from QuEChERS vial
Acetanilide Fraction

Aqueous Fraction

AA Standard

Apple Fruit Amino Acids from the QuEChERS Vial
Acetanilide Fraction

Aqueous Fraction

AA Standard

Add SampliQ AOAC QuEChERS Extraction salt

Add 100 µL extract to autosampler

Spinach extracts were located in the QuEChERS Vial and not used in the AOAC Method 2007.01 or EN 13797.

Peak Area Overlay of eight sequential injections showing reproducibility of the online derivatization and gradient method. Peak area of each amino acid and two trailing minor acids are statistically calculated below. The other amino acids had similar statistics.

Conclusions

• An automated online derivatization method for amino acids using ZORBAX Eclipse Plus C18 was demonstrated as robust by longevity, lot-to-lot reproducibility, and linearity data.
• The Eclipse Plus C18 column choices offer the analyst high resolution, high speed, and reduced solvent consumption, in a combination that best suits one's needs.
• QuEChERS extraction techniques may be useful for analyzing fruit or vegetable for amino acids.
• Fluorescence detection can be substituted for UV detection for higher sensitivity.

References

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