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A rapid LC-MS/MS method for lutein quantification in spinach (*Spinacia oleracea*)

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Appendix A. Supplementary data



Fig. A.1. MS/MS chromatograms of standard lutein (0.4 mg/L) at different column temperature settings: (A) 60 °C, (B) 30°C, and (C) 15°C. All-*trans* lutein (1) and an unknown compound (putative geometrical isomer of lutein) (2) are shown. Arrows indicate the position of the column temperature-dependent unknown peak.



Fig. A.2. Chromatograms of standard lutein (5 mg/L) at a column temperature at 60 °C. The MS/MS chromatogram (A) and UV chromatogram (B) of all-*trans* lutein (1) and an unknown compound (putative geometrical isomer of lutein) (2) are shown, respectively. Arrows indicate each absorbance spectrum of the corresponding peaks in the range from 250 to 800 nm, respectively.

Table A.1. Precursor and product ion *m/z*-values for MS/MS analysis used in the study

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Precursor ion (*m/z*) | Product ion (*m/z*) | Description |
| Lutein | 551.468 | 105.023 | [M-H2O+H]+, quantitative ion |
| Lutein | 568.468 | 105.023 | [M+], confirmation ion |

Table A.2. Comparison of quantitative data obtained from MS/MS detection and in-line UV detection (*n* = 5)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Analyte | Detection technique | Mean (mg 100 g-1) | RSD (%) | *p* value*a* |
| Lutein (sample #1) | MS/MS | 8.98 | 5.07 | 0.52 |
| UV | 8.75 | 8.68 |

*a*Student’s *t*-test between MS/MS detection and UV detection

able A.3. Instruments and gradients used for the inter-laboratory study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Lab no. | LC | MS/MS | Column | *a*Gradient conditions |
| 1 | UltiMate3000 (Thermo Fisher Scientific) | Triple Quad™5500 (SCIEX) | ACQUITY UPLC RBEH C8 (Waters) | 2 solvents |
| 2 | Acquity UPLC H-Class (Waters) | Xevo TQ-S micro (Waters) | ACQUITY UPLC RBEH C8 (Waters) | 3 solvents |
| 3 | Acquity UPLC H-Class (Waters) | Xevo TQ-S micro (Waters) | ACQUITY UPLC RBEH C8 (Waters) | 3 solvents |
| 4 | Acquity UPLC H-Class (Waters) | Xevo TQ-S micro (Waters) | ACQUITY UPLC RBEH C8 (Waters) | 3 solvents |
| 5 | Acquity UPLC H-Class (Waters) | Xevo TQ-S micro (Waters) | ACQUITY UPLC RBEH C8 (Waters) | 3 solvents |

*a*For 2 solvents, mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of acetonitrile/2-propanol (6:1); for 3 solvents, mobile phase A consisted of 0.1% formic acid in water, mobile phase B consisted of acetonitrile, and mobile phase C consisted of 2-propanol.

Separation and elution conditions were described in Materials and Methods.

Table A.4. Calibration curves and regression coefficients used for the inter-laboratory study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Lab no. | *a*Retention time | *b*Range (mg L-1) | Calibration curve | Regression coefficient (*r2*) |
| 1 | 2.72 | 0.0125–0.4 | y = 1322101.1x2 + 532281.15x – 5072.3667 | 0.9999 |
| 2 | 2.02 | 0.0125–0.4 | y = 17162x – 252.19 | 0.9994 |
| 3 | 2.09 | 0.0125–0.4 | y = 22559x – 51.06 | 0.9996 |
| 4 | 1.97 | 0.0125–0.4 | y = 32306x – 218.81 | 0.9994 |
| 5 | 1.98 | 0.0125–0.4 | y = 28288x –199.69 | 0.9988 |

*a*means of 6 points in the calibration curve. *b*6 points in the calibration curve: 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 (mg L-1).

Table A.5. Precursor and product ion *m/z* values of lutein used in the inter-laboratory study

|  |  |  |  |
| --- | --- | --- | --- |
| Lab no. | Precursor ion (*m/z*) | Product ion (*m/z*) | Descriptions |
| 1 | 551.425 | 105.070 | [M-H2O+H]+, quantitative ion |
| 551.425 | 91.055 | [M-H2O+H]+, confirmation ion |
| 551.425 | 119.086 | [M-H2O+H]+, confirmation ion |
| 2 | 551.4 | 105.0 | [M-H2O+H]+, quantitative ion |
| 568.4 | 105.0 | [M+], confirmation ion |
| 3 | 551.400 | 104.800 | [M-H2O+H]+, quantitative ion |
| 4 | 551.468 | 105.023 | [M-H2O+H]+, quantitative ion |
| 568.468 | 105.023 | [M+], confirmation ion |
| 5 | 551.468 | 105.023 | [M-H2O+H]+, quantitative ion |
| 568.468 | 105.023 | [M+], confirmation ion |