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Development of a UPLC-Q-ToF-MS Method for the Determination of Sulforaphane and Iberin in Cruciferous Vegetables

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Cruciferous vegetables

Plant foods belonging to the *Brassicaceae* family and the order of *Brassicales*

The consumption of these vegetables has been correlated with a reduced risk of non-communicable diseases like cancer, diabetes and cardiovascular disease

Common cruciferous vegetables

Manchali et al. *Journal of Functional Foods*, **2012**, *4*, 94-106

Cruciferous vegetables

The health benefits of the cruciferous vegetables can be attributed to the presence of isothiocyanates released after the enzymatic hydrolysis of glucosinolates by myrosinase

Most important isothiocyanates, due to their anti-inflammatory capacity, are **sulphoraphane** (1-isothiocyanato-4-(methylsulfinyl)-butane) and **iberin** (1-isothiocyanato-3-methylsulfinylpropane), produced from the enzymatic hydrolysis of glucoraphanin and glucoiberin glucosinolates

Zhao et al. *European Journal of Pharmacology*, **2018**, *824*, 1-10
Heiss et al. *Journal of Biological Chemistry*, **2001**, *276*, 32008-32015
Subedi et al. *Cells*, **2019**, *8*, 194
Shibata et al. *Journal of Biological Chemistry*, **2014**, *289*, 32757-32772
Wang et al. *Journal of Agricultural and Food Chemistry*, **2005**, *53*, 1417-142

Molecular structures of (a) sulforaphane and (b) iberin

Due to the lack of chromophores, high volatility and precipitation in the liquid chromatography column, the analytical determination of these isothiocyanates is difficult

A very reliable technique for the determination in cruciferous vegetables is the use of **Ultra Performance-Liquid Chromatography-Quadrupole-Time of Flight-Mass Spectrometry (UPLC-Q-ToF-MS)**

Materials

Reagents

-Sulforaphane (synthesized according to D'Souza et al. 2003)
-Iberin
-Dichloromethane (CH_2Cl_2)
-Methanol (LC-MS grade)
-Ultra-pure water (MilliQ purification system)

Standard Solutions

-Sulforaphane and iberin 1000 mg/L in MeOH
-Solution of 10 mg/L in MeOH for the full scan and MS/MS experiments
-Concentrations of 0.1, 0.5, 1.0, 3.0, 5.0, 8.0, 10.0 and 12.0 mg/L by dilution for the construction of calibration curves

D'Souza et al. *Journal of Labelled Compounds and Radiopharmaceuticals*, **2003**, *46*, 851-859

Methods

Samples

-Green broccoli, purple broccoli (sample 1), white cauliflower, white cabbage, red cabbage, watercress and radish from Chalkida, Greece.
-Purple broccoli (sample 2) from Achaia, Greece

Broccoli and cauliflower florets, cabbage and watercress leaves, and roots of radish were used for the preparation of extracts

The samples were lyophilized and ground to a fine homogenous powder using a mortal and pestle

Methods

Preparation of extracts

1. 25 mL of distilled water (pH 7.0) were added into 1 g of dry vegetable
2. Incubation in water bath for 3 hours in 45±3 °C
3. The mixture was left outside for 30 min to reach room temperature
4. 30 mL of CH_2Cl_2 added and stirred for 15 min
5. Filtration using a Buchner funnel with Whatman filter paper grade 1
6. Double extraction of the solid residue with 30 mL CH_2Cl_2
7. Combination of the filtrates into a separation funnel to remove excess water
8. Extract dried with 1 g anhydrous sodium sulfate
9. Evaporation of the solvent to dryness at 35 °C on a Heidolph II rotary evaporator
10. Dissolution of the residue in 1 mL MeOH
11. Injection of the extract to the LC-MS system after a 10-fold dilution with MeOH

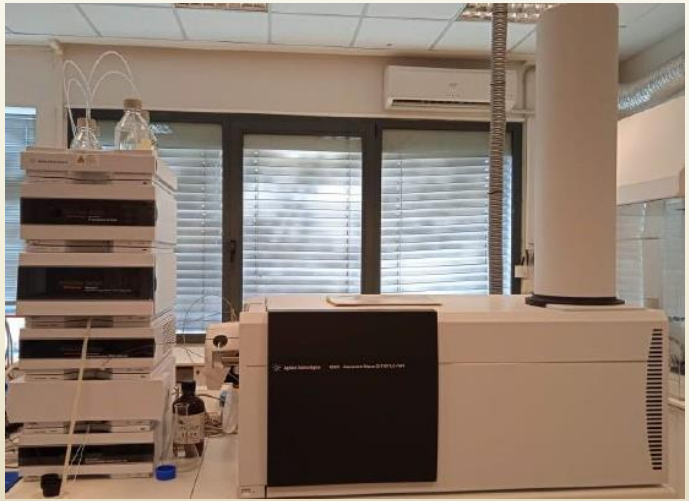
The measurements were performed in triplicates

Campas-Baypoli et al. *Biomedical Chromatography* **2010**, *24*, 387–392

Methods

UPLC-Q-ToF-MS

- The high resolution mass spectrometry spectra were recorded on an Agilent 6530 Quadrupole Time of Flight LC-MS system (Q-ToF-MS), with an ESI source, coupled with Agilent 1290 Infinity UPLC system and an autosampler
- Nitrogen was used as the collision gas and positive electrospray ionization (ESI) was used for the MS experiments
- The data acquisition was carried out with Agilent MassHunter software



Agilent 6530 LC-Q-ToF-MS

Methods

Q-TOF-MS conditions

- Drying gas, 12 L/min
- Gas temperature, 300 ˚C
- Fragmentor, 150V
- Skimmer, 65 V
- Capillary voltage, 4000 V
- Nebulizer gas, 45 psi
- Acquisition rate, 1 spectra/s (threshold 200 Abs, 0.01% rel.)
- MS scan range, 50-1500

Methods

MS/MS experiments

-An auto-MS/MS method was developed with the following parameters: MS/MS acquisition rate, 1 spectra/s (threshold 5 Abs, 0.01% rel.); MS/MS scan range, 50-1500; collision energy slope, 5 V; offset, 2.5 V; preferred charge state, 2, 1, unknown

-The mass accuracy of the Q-ToF-MS was calibrated before each analysis using a calibrant solution for scanning up to m/z 1500

-Mass calibration of the Q-ToF MS was controlled by constant infusion of a reference mass solution into the source of the Q-ToF-MS during the analysis with the reference ions 121.0509 and 922.0098

-The raw data files were processed with Agilent Mass Hunter Qualitative Analysis software

Methods

Chromatographic study

-Performed with an Agilent Zorbax C18 column

-Mobile phase: ultra pure water/0.1% formic acid (A) and MeOH/0.1% formic acid (B)

-Gradient: 0 min: 5% B; 1 min: 5% B; 8.5 min: 95% B; 9.5 min: 95% B; 11.5 min: 5% B; 26.5 min: 5% B

- Total run time including column equilibration: 26.5 min

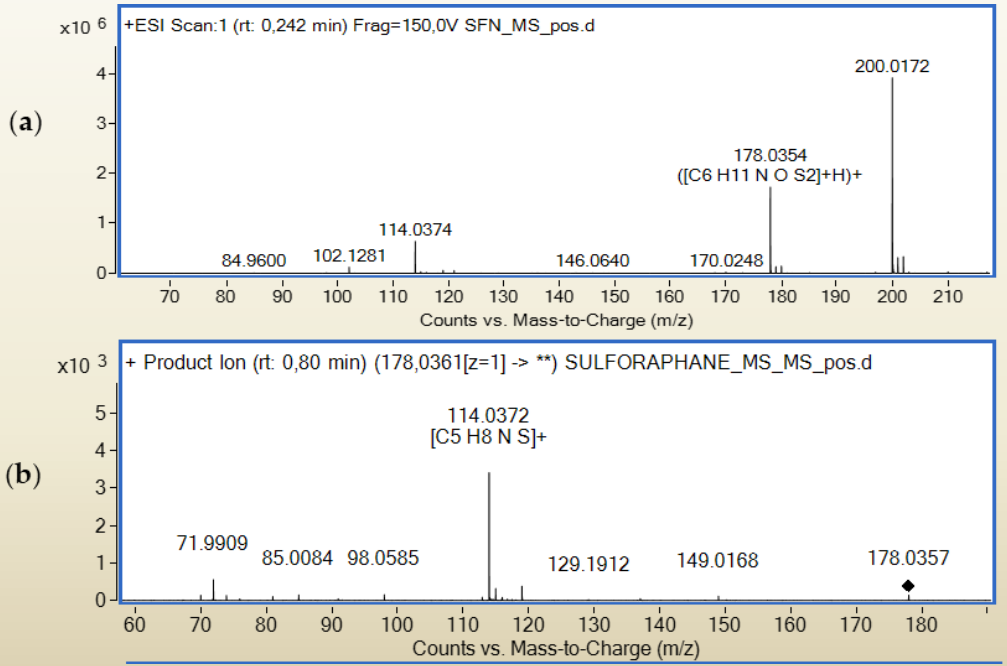
-Injection volume: 2 μL

-Flow rate: 0.4 mL/min

-Column oven temperature: 27 ˚C

Results and discussion

Mass spectrometry study



(**a**) Full scan mass spectrum of sulforaphane $[M+H]^+$ with Δ 0.56 ppm $[M+Na]^+$ with Δ 1.00 ppm

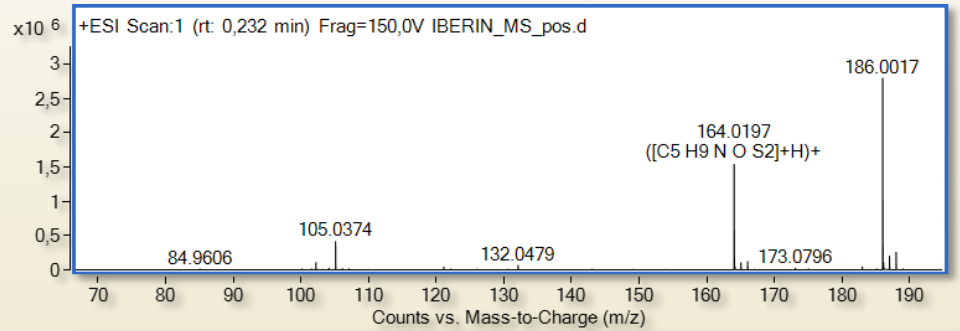
(**b**) MS^2 mass spectrum of sulforaphane

Kokotou et al. *Food Chemistry* **2017**, *237*, 566-573

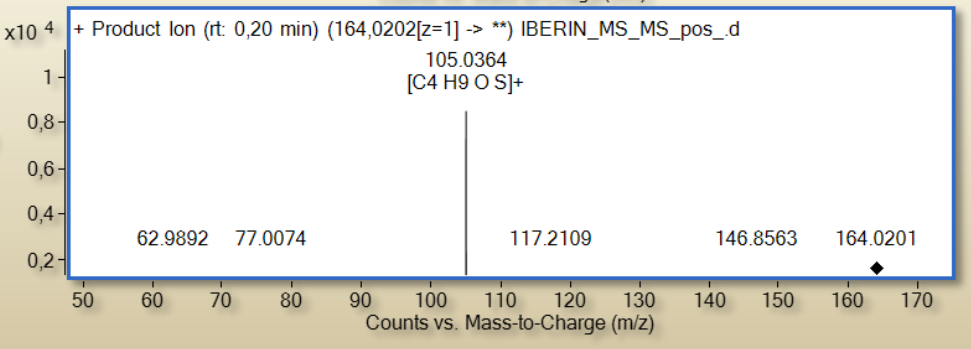
Results and discussion

Mass spectrometry study

(c)



(d)

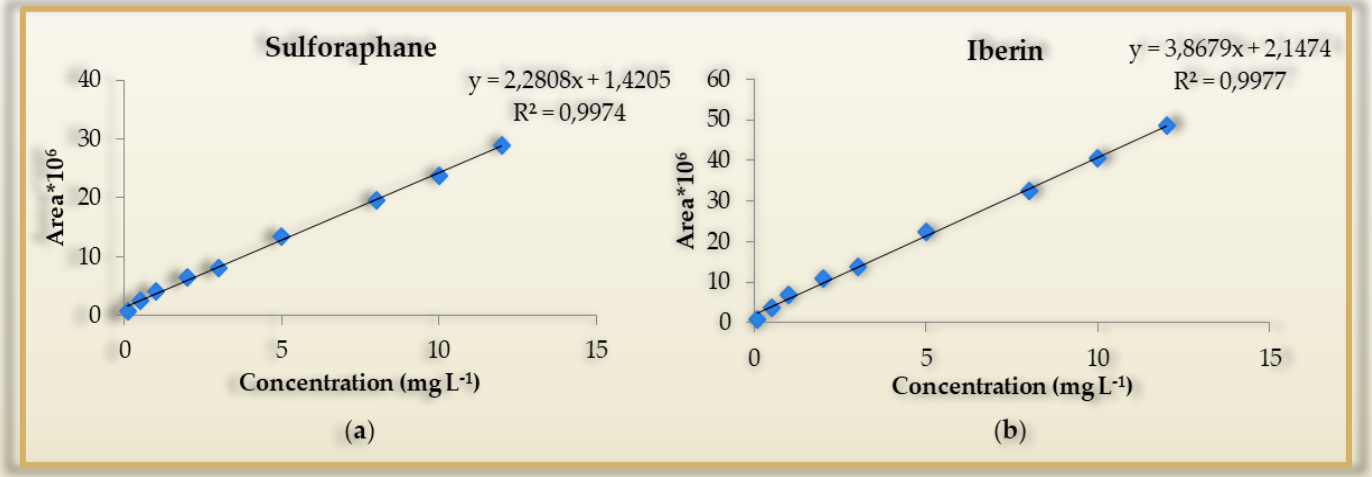


(**c**) Full scan mass spectrum of iberin $[M+H]^+$ with Δ 1.22 ppm $[M+Na]^+$ with Δ 0.54 ppm

(**d**) MS^2 mass spectrum of iberin.

Results and discussion

Method Validation

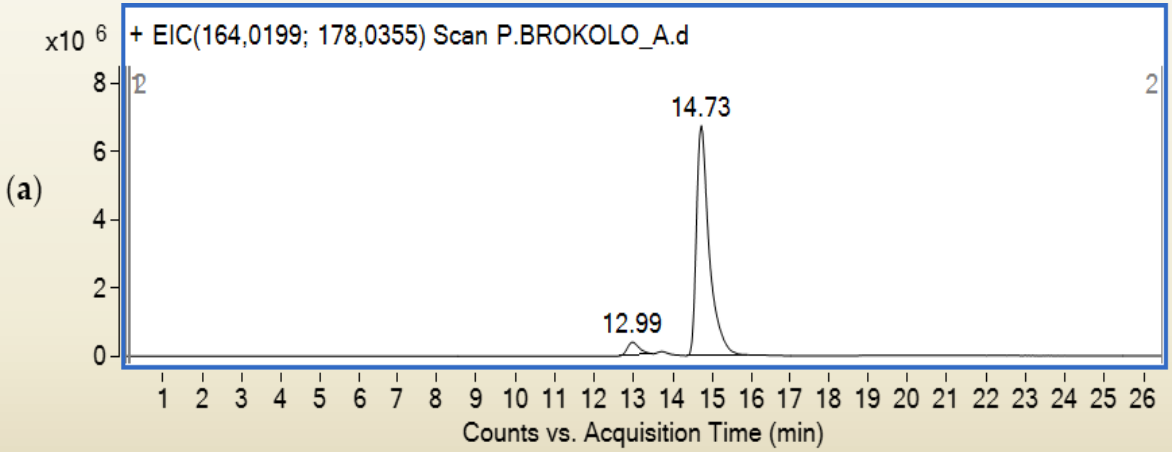


Calibration curves for: (**a**) Sulforaphane; (**b**) Iberin

Limit of detection (LOD) and quantification (LOQ) for **sulforaphane** were 1.19 mg/L and 3.61 mg/L while for **iberin** the LOD and LOQ were calculated at 1.11 mg/L and 3.35 mg/L, respectively

Results and discussion

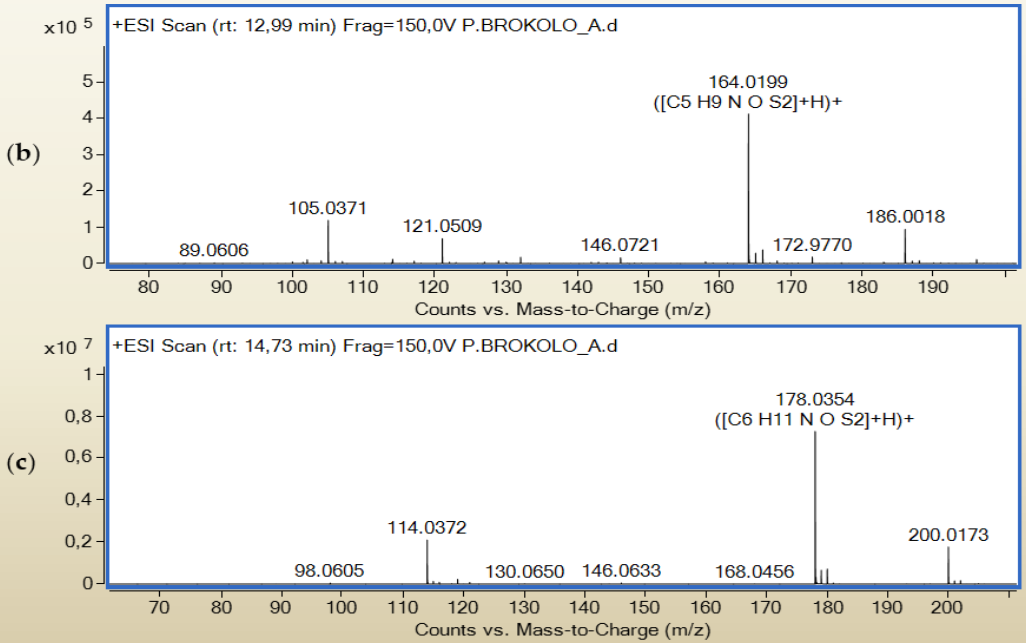
Chromatogram



(**a**) Extracted ion chromatograms of sulforaphane and iberin in green broccoli extract

Results and discussion

Mass spectrometry study



(**b**) Mass spectrum of iberin in green broccoli extract

(**c**) Mass spectrum of sulforaphane in green broccoli extract

Results and discussion

Content of sulforaphane and iberin in various cruciferous vegetables in μg/g dry weight±S.D.

Compound	Green broccoli	Purple broccoli 1	Purple broccoli 2	White cauliflower	White cabbage	Red cabbage	Radish	Watercress
Sulforaphane	660.14±34.29	15.05±0.43	210.11±9.76	14.89±1.62	73.71±1.27	143.83±3.44	9.25±0.14	4.44±0.53
Iberin	20.95±0.67	144.98±3.56	<LOD	47.48±5.07	84.57±0.20	30.12±0.13	0.83±0.09	<LOD

• Green broccoli was found to contain the highest amount of sulforaphane (660.14±34.29 μg/g dry weight)

•The results are in accordance with literature

Karanikolopoulou et al. *Analytica*, **2021**, *2*, 93-120

Conclusions

- A rapid and accurate analytical method for the simultaneous quantification of sulforaphane and iberin in cruciferous vegetables was developed using high resolution mass spectrometry
- The Q-TOF mass analyzer allowed high resolution and accuracy, sensitivity and selectivity, offering rapid and effective food analysis
- The content of sulforaphane and iberin in cruciferous vegetables was in agreement with literature
- To our knowledge, this is the first report employing high resolution mass spectrometry for the simultaneous determination of sulforaphane and iberin in cruciferous vegetables

Thank you for your attention