Journal of Chromatography A, 1348 (2014) 125–136

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Identification and quantification of the main isoflavones and other phytochemicals in soy based nutraceutical products by liquid chromatography–orbitrap high resolution mass spectrometry

Noelia López-Gutiérrez, Roberto Romero-González, Antonia Garrido Frenich*, José Luis Martínez Vidal

Department of Chemistry and Physics (Analytical Chemistry Area), Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería, Agrifood Campus of International Excellence, ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain

ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 11 April 2014
Accepted 28 April 2014
Available online 4 May 2014

Keywords:
Nutraceuticals
Soy
Phytochemicals
Isoflavones
Orbitrap

ABSTRACT

The specific phytochemicals composition of soy nutritional supplements is usually not labelled. Hence, 12 dietary supplements were analyzed in order to detect and identify the main phytochemicals present in these samples, using a database containing 60 compounds. Ultra-high performance liquid chromatography coupled to single-stage Orbitrap high resolution mass spectrometry (UHPLC–Orbitrap–MS) has been used. Two consecutive extractions, using as extraction solvent a mixture of methanol:water (80:20, *v*/*v*), were employed, followed by two dilutions (10 or 100 times depending on the concentration of the components in the sample) with a mixture of an aqueous solution of ammonium acetate 30 mM:methanol (50:50, *v*/*v*). The method was validated, obtaining adequate recovery and precision values. Limits of detection (LODs) and quantification (LOQs) were calculated, ranging from 2 to 150 $\mu g\,L^{-1}$. Isoflavones were the predominant components present in the analyzed supplements with values higher than 93% of the total amount of phytochemicals in all cases. The aglycones (genistein, daidzein, glycitein and biochanin A) as well as their three conjugated forms, β-glucosides (genistin, daizin and glycitin) were detected and quantified, being daidzein the isoflavone detected at higher concentration in 8 out of 12 samples reported, with values ranging from 684 to 35,970 $mg\,kg^{-1}$, whereas biochanin A was detected at very low concentrations, ranging from 18 to 50 $mg\,kg^{-1}$. Moreover, other phytochemicals as flavones, flavonols, flavanones and phenolic acids were also detected and quantified.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The large amount of bioactive substances present in plants has contributed to the appearance of nutraceutical products, which are considered as a food, or part of a food, that provide medicinal or health benefits [1], including the prevention and treatment of disease attributed to their bioactive ingredients, the phytochemicals [2].

The main substances present in soy supplements are the isoflavones, which are classified as phytoestrogens [3], and they are structurally similar to the mammalian oestrogen, oestradiol-17β, and exhibit oestrogenic properties [4]. Bearing in mind some side effects detected when "classical" hormones were used [5], soy dietary supplements were used as complementary therapy [6] although there are controversies about their effectiveness [7,8].

Concerning other biomedical properties, these natural products have been widely used as responsible of health-promoting benefits, especially associated with the prevention of cardiovascular disease [9], diabetes and obesity [10,11] and prostate cancer [12]. These health benefits are mainly associated with the active part of the isoflavone molecule (aglycone without the sugar moiety) [3,13].

In soy based products, the principal isoflavones are aglycons [14], as genistein (0.15–9.98 $mg\,g^{-1}$), daidzein (0.83–40.78 $mg\,g^{-1}$) and glycitein (0.22–0.49 $mg\,g^{-1}$) [15,16]. These compounds may also be present as their conjugated forms, as the β-glucoside form (genistin, daidzin, and glycitin), the acetylglucoside form (6″-*O*-acetylgenistein, 6″-*O*-acetyldaidzin, and 6″-*O*-acetylglycitin), and the malonylglucoside form (6″-*O*-malonylgenistin, 6″-*O*-malonyldaidzin, and 6″-*O*-malonylglycitin) [17,18]. The total concentration is normally expressed as aglycones equivalents and it is usually ranged from 0.40 [19] to 365 $mg\,g^{-1}$ [20] in dietary supplements.

* Corresponding author. Tel.: +0034 950015985; fax: +0034 950015008/+34 950015483.
E-mail address: agarrido@ual.es (A. Garrido Frenich).

http://dx.doi.org/10.1016/j.chroma.2014.04.090
0021-9673/© 2014 Elsevier B.V. All rights reserved.

In order to correctly evaluate the biological effects of isoflavones, it is necessary to screen the different types of isoflavones, and accurate quantification of these compounds in their original forms [17]. Moreover, and bearing in mind that dietary supplements are often blends of herbal extracts containing other or unknown compounds [21], it is necessary to ensure the quality of these products in terms of identification and determination of bioactive compounds [22].

In general, there are two strategies for isoflavones analysis. Thus, the analysis of native conjugated isoflavones profiles can be performed [23,24], although an hydrolysis step can be carried out in order to determine the isoflavones in their aglycone form. For this last purpose, three types of hydrolysis, acid, basic or enzymatic, could be used [21,25,26].

The most common methods for the extraction of the isoflavones from solid samples include a solid–liquid extraction using as extraction solvent a mixture of methanol:water (80:20, *v*/*v*) [26,27], ethanol at different percentages (from 70 to 96%) [16,28] and acetonitrile, which can be used at different proportions depending on the polarity of the target compounds [17].

In relation to the determination of isoflavones in soybean, soy derived or nutraceutical products, the analytical methods are mainly based on liquid chromatography (LC) coupled with UV [23,29], diode array detection (DAD) [4,16,25] or mass spectrometry (MS) [30–33].

However, high resolution MS (HRMS) has been scarcely applied for the determination of isoflavones and other bioactive compounds in nutraceutical products, although its efficacy has previously been demonstrated in others matrices as juice [34] or sofrito [35]. HRMS can provide several advantages in relation to conventional analyzers as triple quadrupole (QqQ), such as full scan acquisition mode, with no limitations in the number of monitored compounds, all information can be obtained in a single injection and retrospective analysis could be performed. Moreover, exact mass measurements can also be helpful to improve the knowledge on the composition of these products. Therefore, in addition to the compounds most commonly identified in soy based products (isoflavones) other phytochemicals can be identified and/or quantified, increasing the scope of the analysis.

The aim of this research is the utilization of this technique (ultra high performance LC (UHPLC) coupled to Orbitrap–MS) for the development and validation of an analytical method, which allows the quantification and determination of several types of isoflavones and other phytochemicals as flavones, flavonols, flavanones or phenolic acids in commercial soy-based nutraceutical products (tablets and capsules).

2. Experimental

2.1. Chemicals and reagents

All phytochemicals standards were purchased from Extrasynthese (Genay, France), Sigma-Aldrich (Madrid, Spain), ChromaDEX (Irvine, CA, USA) and Fluka (Steinheim, Germany). All standards have a purity > 95%, except quercetin-3-*O*-glucoside with a purity ≥90%. Caffeine–$^{13}C_3$ solution (1 mg L^{-1}) was obtained from Sigma-Aldrich. Individual stock standard solutions were prepared by dissolving the powder in methanol, ethanol, dimethyl sulfoxide or in a mixture of methanol:water (50:50 *v*/*v*), ranging from 90 to 1350 mg L^{-1}. These stock solutions were kept in amber bottles, and they were stored at −18 °C in the darkness for no longer than 6 months.

A multi-compound working solution (5 mg L^{-1}) was prepared by appropriate dilution of aliquots of each individual stock standard solution with methanol, and it was stored under refrigeration (<−18 °C) in an amber bottle in the dark for 6 months.

Acetonitrile (ACN) (LC–MS grade) was supplied by Fisher Scientific (Fair Lawn, NJ, USA). Methanol (LC–MS grade) and dimethyl sulfoxide (HPLC grade) were purchased from Sigma-Aldrich. Water (LC–MS grade) and formic acid (LC–MS grade) were supplied by Scharlau (Barcelona, Spain). Ethanol (HPLC grade) and ammonium acetate (purity 97%) was supplied by Panreac (Barcelona, Spain). Hydrochloric acid was purchased from J.T. Baker (Deventer, The Netherlands). In order to accurate mass calibration, a mixture of acetic acid, caffeine, Met–Arg–Phe–Ala–acetate salt and Ultramark 1621 (ProteoMass LTQ/FT-hybrid ESI positive), and a mixture of acetic acid, sodium dodecyl sulfate, taurocholic acid sodium salt hydrat and Ultramark 1621 (fluorinated phosphazines) (ProteoMass LTQ/FT-Hybrid ESI negative) from Thermo-Fisher (Waltham, MA, USA) were employed in the Orbitrap analyzer.

Millipore Millex-LG filters (0.20 μm, Millipore, Carrightwohill, Ireland) were used to filtrate the extracts.

2.2. Apparatus

The extraction method was performed using a Centronic BL II centrifuge (J.P. Selecta, Barcelona, Spain), a Reax 2 rotatory agitator from Heidolph (Schwabach, Germany), and vortex mixer WX from Velp Scientifica (Usmate, Italy). A coffee grinder (Orbit, Hong Kong, China) was used to process all samples.

2.3. UHPLC–Orbitrap–MS analysis

An UHPLC system Transcend (Transcend 600 LC, Thermo Fisher Scientific, San Jose, CA, USA) was employed for chromatographic analysis. A Waters (Milford, MA, USA) Acquity C18 column (2.1 × 100 mm, 1.7 μm particle size) was used for the separation of the compounds.

A single-stage Orbitrap mass spectrometer (Exactive™, Thermo Fisher Scientific, Bremen, Germany) was used for MS analyses. The instrument was operated using a heated electrospray interface (ESI) (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA), in positive (ESI+) and negative ionization (ESI−) modes. ESI parameters were as follows: spray voltage, 4 kV (−4 kV in ESI−); sheath gas (N_2, >95%), 35 (adimensional); auxiliary gas (N_2, >95%), 10 (adimensional); skimmer voltage, 18 V (−18 V in ESI−); capillary voltage, 35 V (−35 V in ESI−); tube lens voltage, 95 V (−95 V in ESI−); heater temperature, 305 °C; capillary temperature, 300 °C. The automatic gain control (AGC) was set at a target value of 1×10^6. The mass spectra were acquired employing four alternating acquisition functions: (1) full MS, ESI+, without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power = 25,000 FWHM; scan time = 0.25 s; (2) All-ion fragmentation (AIF), ESI+, with fragmentation (HCD on, collision energy = 30 eV), mass resolving power = 10,000 FWHM; scan time = 0.10 s; (3) full MS, ESI− without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power = 25,000 FWHM; scan time = 0.25 s; (4) AIF, ESI−, with fragmentation (HCD on, collision energy = 30 eV), mass resolving power = 10,000 FWHM; scan time = 0.10 s. Considering the scan time of the four acquisition functions, and the polarity switching (approx. 0.27 s), an overall scan rate of 0.56 Hz was achieved. Mass range in the full scan experiments was set at *m*/*z* 100 to 1000, whereas for MS/MS, it was set from *m*/*z* 70 to 700.

All the analyses were performed without lock mass, using external calibration mode. Mass accuracy was checked with multi-compound standards every day and the analyzer was calibrated every two weeks with mass accuracy standards (see Section 2.1). Data acquisition and processing were carried out using Xcalibur™ version 2.2.1 (Thermo Fisher Scientific, Les Ulis, France) with Qual and Quan browser. ICIS peak detection was applied. ToxID™ 2.1.1

Download English Version:

https://daneshyari.com/en/article/1200245

Download Persian Version:

https://daneshyari.com/article/1200245

Daneshyari.com