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Method development and validation for isoflavones in soy germ pharmaceutical capsules using micellar electrokinetic chromatography

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Abstract

The separation of six soy isoflavones (Glycitein, Daidzein, Genistein, Daidzin, Glycitin and Genistin) was approached by a 3^2 factorial design studying MEKC electrolyte components at the following levels: methanol (MeOH; 0–10%) and sodium dodecylsulfate (SDS; 20–70 mmol L^{-1}); sodium tetraborate buffer (STB) concentration was kept constant at 10 mmol L^{-1}. Nine experiments were performed and the apparent mobility of each isoflavone was computed as a function of the electrolyte composition. A novel response function (RF) was formulated based on the productory of the mobility differences, mobility of the first and last eluting peaks and the electrolyte conductance. The inspection of the response surface indicated an optimum electrolyte composition as 10 mmol L^{-1} STB (pH 9.3) containing 40 mmol L^{-1} SDS and 1% MeOH promoting baseline separation of all isoflavones in less than 7.5 min.

The proposed method was applied to the determination of total isoflavones in soy germ capsules from four different pharmaceutical laboratories. A 2 h extraction procedure with 80% (v/v) MeOH under vortexing at room temperature was employed. Peak assignment of unknown isoflavones in certain samples was assisted by hydrolysis procedures, migration behavior and UV spectra comparison. Three malonyl isoflavone derivatives were tentatively assigned. A few figures of merit for the proposed method include: repeatability ($n = 6$) better than 0.30% CV (migration time) and 1.7% CV (peak area); intermediate precision ($n = 18$) better than 6.2% CV (concentration); recoveries at two concentration levels, 20 and 50 μg mL^{-1}, varied from 99.1 to 103.6%. Furthermore, the proposed method exhibited linearity in the concentration range of 1.6–50 μg mL^{-1} ($r^2 > 0.9999$) with LOQ varying from 0.67 to 1.2 μg mL^{-1}. The capsules purity varied from 93.3 to 97.6%.

Keywords: Isoflavone; Soy (*Glycine max* L.); Capillary electrophoresis; Genistein; Daidzein

1. Introduction

Isoflavones are phytochemical secondary metabolites belonging to the flavonoids group found in a variety of plants, especially soy (*Glycine max*), with potential benefits for human health [1]. Soy products have been extensively investigated and their systematic consumption has been associated to the prevention of certain cancers [2], retardation of bone loss and risk for osteoporosis [3], reduction of cardiovascular disease risk factors [4], menopausal symptoms, among other activities. Due to the fact that isoflavones structurally or functionally mimic mammalian estrogens, they are often referred as phytoestrogens, although many of their benefits are being attributed to metabolic properties other than estrogen receptor interactions, such as influence on enzymes, protein synthesis, calcium transport, cell proliferation and differentiation, etc. [5] The structural features of the soy isoflavones under consideration in this work are presented in Fig. 1.

The determination of isoflavones in food and biological specimens has been performed traditionally by chromatographic techniques, UV and IR spectroscopy and immunoassays [5–7]. Gas chromatography coupled to mass spectrometry [8,9], high-performance liquid chromatography (HPLC) with UV [10–13], electrochemical [14–16] and mass spectrometry [17–19] detec-

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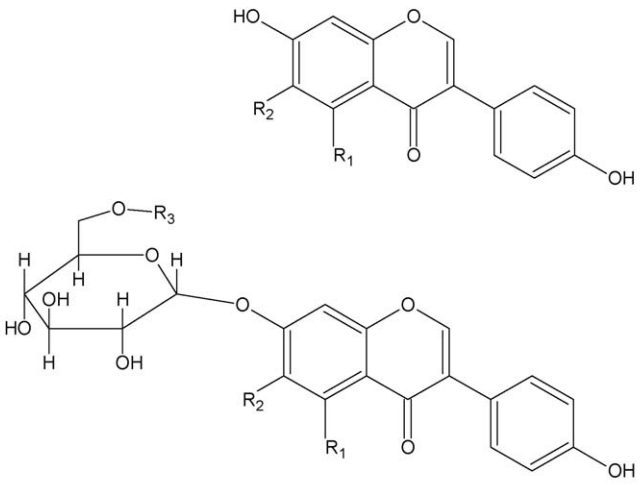

COMPOUND	ISOFLAVONE	Abbreviation	R_1	R_2	R_3
1	Glycitein	Gly	H	OCH3	-
2	Daidzein	De	H	H	-
3	Genistein	Ge	OH	H	-
4	Daidzin	Din	H	H	H
5	Glycitin	Glyn	H	OCH3	H
6	Genistin	Gin	OH	H	H
7	6′′-Malonyldaidzin	Mdin	H	H	$COCH_2COOH$
8	6′′-Malonylglycitin	Mglyn	H	OCH3	$COCH_2COOH$
9	6′′-Malonylgenistin	Mgin	OH	H	$COCH_2COOH$
10	6′′-O-Acetyldaidzin	Adin	H	H	$COCH_3$
11	6′′-O-Acetylglycitin	Aglyn	H	OCH3	$COCH_3$
12	6′′-O-Acetylgenistin	Agin	OH	H	$COCH_3$

Fig. 1. Structural features of the soy isoflavones.

tion are among the most commonly employed techniques for the determination of isoflavones and other phytoestrogens in soy extracts, soy milk, soy based infant food and nutrition soy based supplements. As an alternative technique, capillary electrophoresis has been selected occasionally for the analysis of phytoestrogens and isoflavones in biological materials [20], soy seeds [21] and plants [22], soy based products [23], as well as other medicinal preparations [24–26]. The ionization constant of five isoflavones has been determined by McLeod and Shepherd [27]. Except for the work of Novotny and coworkers [20], who employed capillary electrochromatography (CEC), all other isoflavone separations have been approached by the capillary zone electrophoresis mode (CZE).

In this work, the separation of six isoflavones (three aglycones and three glycosides) was attempted by a micellar electrokinetic chromatography (MEKC) methodology using a 3^2 factorial designed electrolyte composed of tetraborate/SDS and modified by methanol. The method was validated and further applied in the determination of isoflavones in soy germ pharmaceutical capsules.

2. Experimental

2.1. Instrumentation

Direct detection experiments were conducted in a capillary electrophoresis system (model $HP^{3D}CE$, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector set at 269 nm, a temperature control device maintained at 20 °C and an acquisition and data treatment software supplied by the manufacturer (HP ChemStation, rev. A.06.01). Samples were injected hydrodynamically (40 mbar for 3 s; 1 mbar = 100 Pa) and the system was operated under normal polarity of +25 kV.

Indirect detection experiments were conducted in a capillary electrophoresis system (model P/ACE 5510, Beckman Instruments, Fullerton, CA, USA) equipped with a filter-carrousel UV detector positioned at the 254 nm filter, a temperature control device maintained at 25 °C and an acquisition and data treatment software supplied by the manufacturer (Beckman P/ACE System Gold® Software). Samples were injected hydrodynamically (0.5 psi for 3 s; 1 psi = 6.8927 kPa) and the system was operated under inverted polarity of −20 kV.

Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with dimensions 58.5 cm total length, 50.0 cm effective length and 75 μm i.d. and 375 μm o.d. were used. At the beginning of each day, the capillary was conditioned by a flushing of 1 mol L^{-1} NaOH (5 min) followed by a 5 min flush of deionized water and electrolyte solution (30 min). In between runs, the capillary was reconditioned with the electrolyte solution (3 min flush). Flushing pressures were 930 mbar and 20 psi for the Agilent and Beckman equipments, respectively.

2.2. Reagents and solutions

All reagents were of analytical grade, solvents were of chromatographic purity and the water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA).

Stock solutions of six isoflavone standards (Daidzein, Daidzin, Genistein, Genistin, Glycitein and Glycitin) obtained from ChromaDex (Santa Ana, CA, USA) were prepared in methanol at 200 mg L^{-1} concentration and stored at 4 °C until analysis.

In the direct analysis of isoflavones an optimal electrolyte composed of 10 mmol L^{-1} sodium tetraborate (STB) at pH 9.3, 40 mmol L^{-1} sodium dodecylsulfate (SDS) and 1% (v/v) methanol (MeOH) was used.

In the indirect analysis of carboxylic acids an electrolyte composed of 10 mmol L^{-1} 3,5-dinitrobenzoic acid adjusted at pH 3.55 with diluted NaOH containing 0.5 mmol L^{-1} cetyltrimethylammonium bromide (CTAB) was used.

2.3. Samples

Soy germ pharmaceutical capsules were acquired in local drug stores from four different Brazilian manufacturers. The capsules contained dry extracts of soy, *G. max* L. (Laboratory A) or dry extracts of soy dispersed in powder excipients (silicon dioxide, magnesium stearate or silicate and microcrystalline cellulose; Laboratories B and C) or suspended in soy oil (Laboratory D). The contents of ten capsules were mixed and weighed (50 mg) to separate vials. The isoflavones were extracted by 2.00 mL of a methanolic aqueous solution (80%, v/v, MeOH) under vortexing at room temperature for 2 h. The extracts were centrifuged and to the uppernatant a 80 mmol L^{-1} STB solution and water were added (1:1:6, v/v/v). The diluted solution was injected in the CE system. For the peak assignment studies, exactly 1.60 mL of the uppernatant were taken and loaded to a solid-phase extraction octadecyl bonded silica cartridge (SPE Strata C18-E, 50 μm, 70 Å, 1000 mg/6 mL, Phenomenex Inc., Torrance, CA, USA) preconditioned with 6.00 mL of MeOH and rinsed with 6.00 mL water. After sample loading, the cartridge was rinsed with 3.00 mL water (discharged fraction) followed by 1.20 mL MeOH (collected fraction). To the collected fraction, a 100 mmol L^{-1} STB solution and water were added (1:1:8, v/v/v). This later fraction was divided into two aliquots: one was reserved for the direct analysis of isoflavones and indirect analysis of carboxylic acids. The second aliquot was submitted to hydrolysis in a water bath at 80 °C temperature for 30 min, prior to injection.

Table 1
3^2 factorial design levels

Run	MeOH	SDS
1	−	−
2	−	0
3	−	+
4	0	−
5	0	0
6	0	+
7	+	−
8	+	0
9	+	+

Levels—MeOH: 0% (−), 5% (0) and 10% (+); SDS: 20 mmol L^{-1} (−), 40 mmol L^{-1} (0) and 70 mmol L^{-1} (+).

3. Results and discussion

3.1. Optimization of the separation

Preliminary CZE experiments have shown that pH has little effect on the mobility of the isoflavone glycosides. Since only three out of the twelve soy isoflavones under consideration in this work are aglycones (Fig. 1), the separation was attempted in micellar medium and solvent effects and SDS variation were investigated as the most probable variables affecting selectivity.

The optimization of the separation of the isoflavone standards 1–6 (Fig. 1) was approached by a 3^2 factorial design studying the electrolyte components at the following levels: MeOH (0–10%), SDS (20–70 mmol L^{-1}); the STB concentration was kept constant at 10 mmol L^{-1}. Nine experiments were performed (Table 1) and the apparent mobility of each isoflavone analyte was computed as a function of the electrolyte composition according to the following empirical equation:

$$\mu_i = \text{constant} + a[\text{MeOH}] + b[\text{SDS}] + c[\text{MeOH}]^2 + d[\text{SDS}]^2 + e[\text{MeOH}][\text{SDS}] \quad (1)$$

where μ_i is the apparent mobility of isoflavone i. The equations were solved numerically by means of the Solver algorithm (Microsoft® Excel 2000, version 9.0.2812) and the coefficients are organized in Table 2.

Table 2
Coefficients of the mobility function (Eq. (1))

Isoflavone[a]	Constant	a	b	c	d	e
1	18.28	−1.69	0.43	0.050	−0.0020	−0.0023
2	25.74	−1.25	0.09	0.046	0.000088	0.0000
3	25.52	−1.09	0.06	0.033	0.00040	0.0000
4	24.27	−1.80	0.36	0.046	−0.0018	0.0017
5	22.43	−2.04	0.41	0.059	−0.0023	0.0015
6	21.03	−0.80	0.15	0.027	−0.0066	−0.0028

[a] Numbering according to Fig. 1.

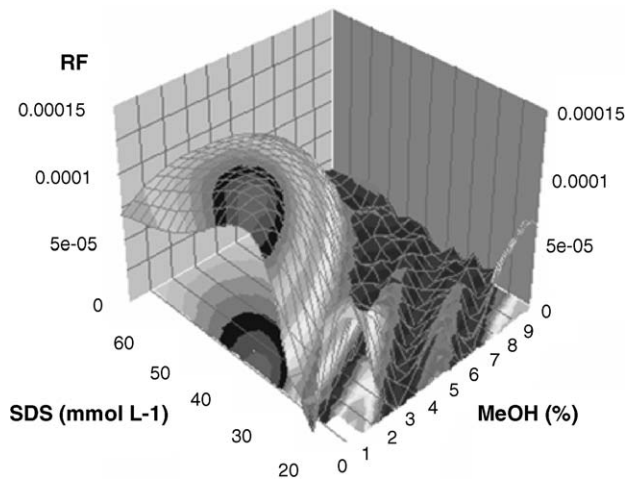

Fig. 2. Response surface based on the novel response function from Eq. (2).

A novel response function (RF) was formulated according to the expression:

$$\mathrm{RF} = \frac{\prod_{i=1}^{n}(\mu_{i+1} - \mu_i)}{\mu_{\mathrm{last}} - \mu_{\mathrm{first}}} \mu_{\mathrm{last}}^{-2} G^{-2} \quad (2)$$

where μ_i is the mobility of isoflavone i of n, given by Eq. (1), $\prod$ the productory of the mobility differences, μ_{last} and μ_{first} the mobilities of the last and first eluting peaks, respectively, and G is the electrolyte conductance (measured current over the applied voltage).

The first term of Eq. (2) is a measure of the system overall resolution, i.e. the larger the value of $\prod$ the larger the resolution of all adjacent solute pairs. $\prod$ is divided by the mobility interval ($\mu_{\mathrm{last}} - \mu_{\mathrm{first}}$) because resolution depends on the mobility magnitude. The term ($\mu_{\mathrm{last}}^{-2} G^{-2}$) is related to the analysis time. Since the analysis is conducted under counter-electroomostic flow, the larger the analyte mobility, the slower the analyte. Likewise the smaller the value of G, the larger the electroosmotic flow magnitude and consequently the smaller the analyte migration time. To electrolytes of low conductance a larger voltage can be applied. In the studied system, Ohm's law held up to 85 μA.

The response function (Eq. (2)) was calculated for the entire dataset and a response surface was generated (Fig. 2) indicating optimum conditions for separation with an electrolyte composed of 40 mmol L^{-1} SDS and 1% MeOH (fixed STB at 10 mmol L^{-1} concentration).

3.2. Optimization of the sample diluent

The corresponding electropherogram of a solution of isoflavone standards at the optimal condition is depicted in Fig. 3. When the standard solution is prepared in pure methanol, peaks 1–3 are thinner and fairly symmetrical whereas peaks 4–6 are broader and fronting (Fig. 3A). This behavior might be explained by the fact that a possible demicellization process occurs at the methanol rich sample plug. Therefore, SDS monomers or aggregates should carry the analytes towards the anodic electrolyte region in a much slower pace than completely assembled micelles would do. Since solutes 1–3 are ionized in both sample plug and electrolyte medium, such solutes undergo stacking and migrate out of the sample plug much faster than the solutes 4–6.

Several other sample diluents were tested: SDS solutions from 20 to 35 mmol L^{-1} with and without STB buffer and varied amounts of methanol (<100%). The best results in terms of peak symmetry and efficiency were obtained when the standards were dissolved in 10 mmol L^{-1} STB containing 10% MeOH (Fig. 3B). On-line acquired UV spectra are depicted alongside Fig. 3.

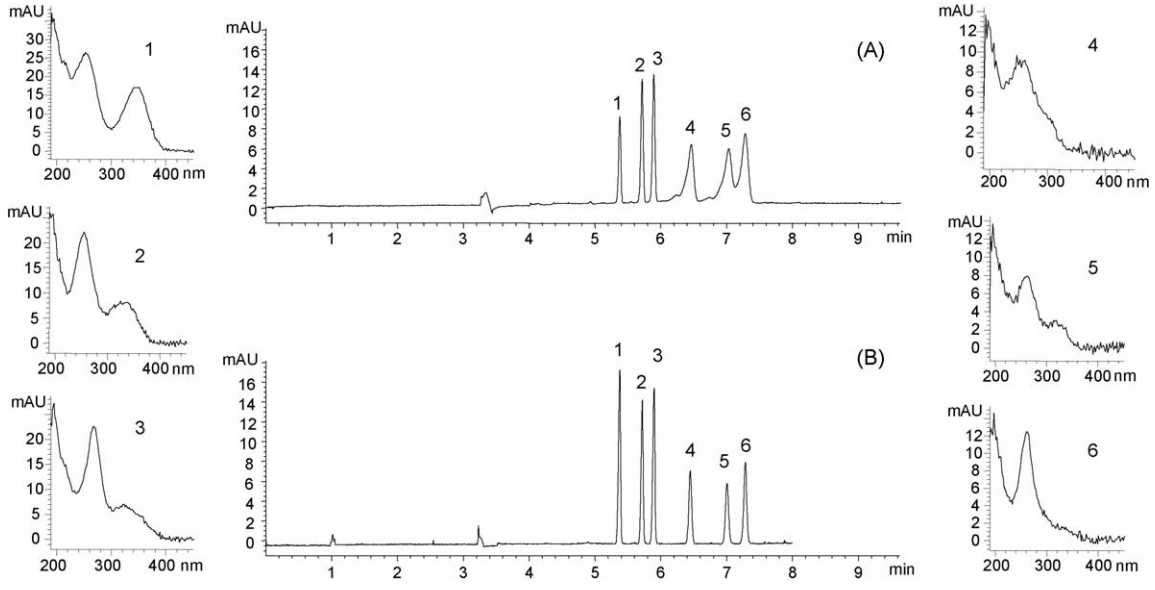

Fig. 3. Optimization of sample diluent. Electropherogram of isoflavone standards diluted in: (A) methanol and (B) 10 mmol L^{-1} STB and 10% MeOH. Separation conditions: 10 mmol L^{-1} STB (pH 9.3), 40 mmol L^{-1} SDS and 1% MeOH; injection, 40 mbar, 3 s; applied voltage, +25 kV; 58.5 cm × 75 μm i.d. capillary; 20 °C; direct detection, 269 nm. Peak labels as in Fig. 1. Lateral figure: on-line acquired UV spectra of the corresponding numbered peaks.

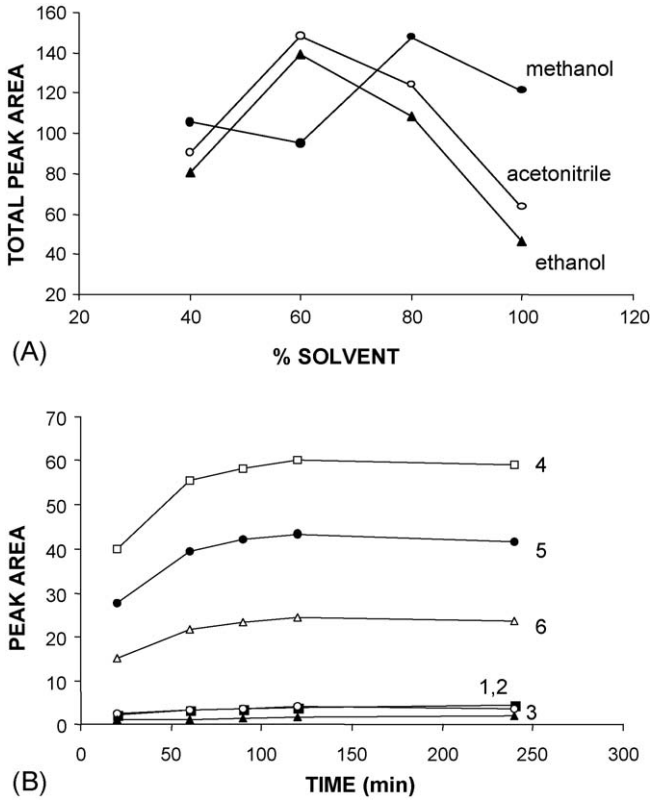

Fig. 4. Optimization of the extraction procedure for isoflavones in soy germ capsules. (A) Effect of solvent type for 2 h extraction period and (B) effect of extraction time for 80% MeOH as extraction solvent. Separation conditions: 10 mmol L^{-1} STB (pH 9.3), 40 mmol L^{-1} SDS and 1% MeOH; injection, 40 mbar, 3 s; applied voltage, +25 kV; 58.5 cm × 75 μm i.d. capillary; 20 °C; direct detection, 269 nm. Curve labels as in Fig. 1.

3.3. Optimization of the sample extraction procedure

Fig. 4A presents the total amount of isoflavones extracted from a soy germ capsule sample by increasing amounts of different solvents (methanol, ethanol and acetonitrile). As it can be seen, higher amounts of isoflavones were extracted by either 60% acetonitrile or 80% MeOH. These results are in good agreement with the literature [28–31]. A few authors acidified the sample prior to extraction to achieve higher yields [32]. Others employ alternative extraction procedures such as ultrasound [33] and supercritical fluid extraction [34]. In this work, an 80% methanolic solution was selected for extraction because methanol was already part of the electrolyte medium. Furthermore, the use of high percentages of methanol minimizes the concomitant extraction of soy proteins, which could be detrimental to the analysis due to protein adsorption to the capillary wall, altering the electroosmotic flow magnitude.

In Fig. 4B, the extraction time was investigated using 80% MeOH as extractor solution. As it can be observed, above ≈80 min there was no significant increase in the extracted amount of isoflavones. An extraction time of 2 h was then selected for further studies.

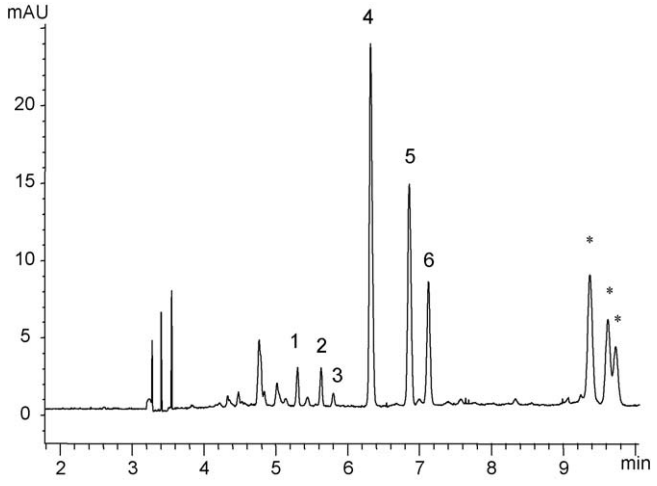

Fig. 5. Optimal condition for the separation of isoflavones in soy germ capsules. Sample extracted in 80% MeOH for 2 h. Separation conditions: 10 mmol L^{-1} STB (pH 9.3), 40 mmol L^{-1} SDS and 1% MeOH; injection, 40 mbar, 3 s; applied voltage, +25 kV; 58.5 cm × 75 μm i.d. capillary; 20 °C; direct detection, 269 nm. Peak labels as in Fig. 1; (*) unknown.

3.4. Tentative identification of sample components

From the twelve isoflavones that have been identified in soy (Fig. 1), the malonyl derivatives are expected to migrate the slowest in normal MEKC due to the presence of the carboxylate group.

Fig. 5 presents the electropherogram of a typical soy germ extract under the optimized conditions. Peaks were identified by spiking procedures with standard solutions and by comparison with UV spectra library. Based on the electrophoretic behavior and UV spectra, the group of peaks marked with asterisks appearing at longer migration time was tentatively attributed to malonyl isoflavone derivatives.

According to Carrão-Panizzi et al. [35] malonyl derivatives are readily converted to acetyl derivatives or to the corresponding glycosides upon hydrolysis. Furthermore, free malonic acid is not expected to be present due to decarboxylation reaction under high temperature forming acetic acid and CO_2 [36].

In order to provide a further evidence for the tentative peak assignment of malonyl isoflavones, a soy germ sample extract was cleaned by solid-phase extraction in octadecyl-silica cartridge (removal of naturally occurring acetic acid) and hydrolyzed at 80 °C for 30 min. The separation conditions (increase of SDS to 50 mmol L^{-1} and MeOH to 10% in the electrolyte) were modified to improve resolution of the last eluting peaks. As it can be visualized in the electropherograms of Fig. 6, the peaks attributed to malonyl derivatives (peaks 7–9; previously marked in Fig. 5 with asterisks) disappeared upon hydrolysis whereas the corresponding glycosides (peaks 4–6) increased slightly. Additionally, the isoflavone glycoside and its corresponding malonyl derivative, i.e. compound pairs 4 and 7, 5 and 8 as well as 6 and 9 present spectral similarities (compare UV spectra alongside Figs. 3 and 6). Furthermore, the absence of acetate in the cleaned sample extract and appearance of acetate in the hydrolyzed sample (Fig. 7) supports the decar-

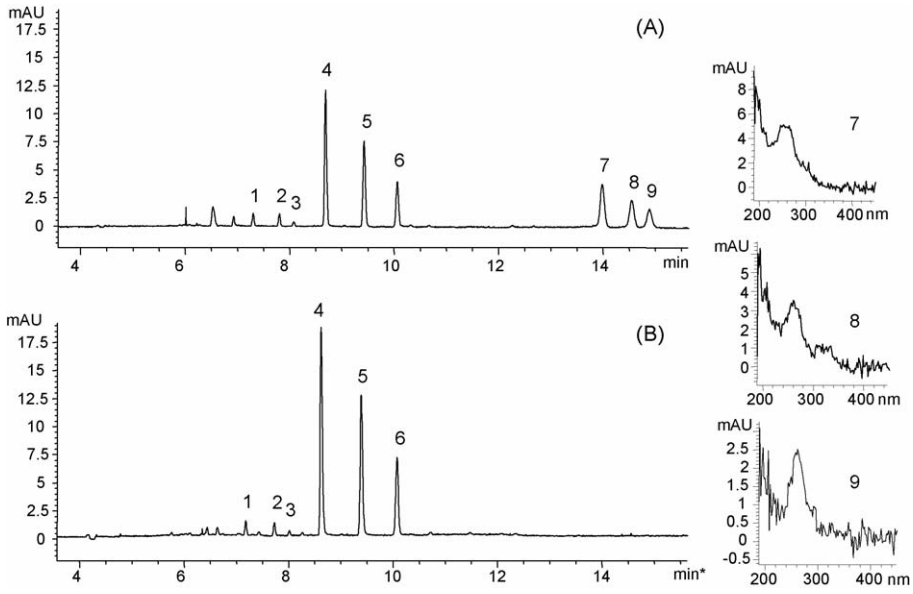

Fig. 6. Hydrolysis studies: analysis of isoflavones. Soy germ capsules extracts prepared at 80% MeOH for 2 h and submitted to solid-phase extraction in octadecylsilica cartridges (A) followed by hydrolysis at 80 °C for 30 min (B). Separation conditions: 10 mmol L^{-1} STB (pH 9.3), 50 mmol L^{-1} SDS and 10% MeOH; injection, 40 mbar, 3 s; applied voltage, +25 kV; 58.5 cm × 75 μm i.d. capillary; 20 °C; direct detection, 269 nm. Peak labels as in Fig. 1. Lateral figure: on-line acquired UV spectra of the corresponding numbered peaks.

boxylation of malonic acid and further sustains the previous peak assignment.

3.5. Method validation

In order to validate the proposed methodology for inspection of isoflavone contents in soy germ pharmaceutical capsules

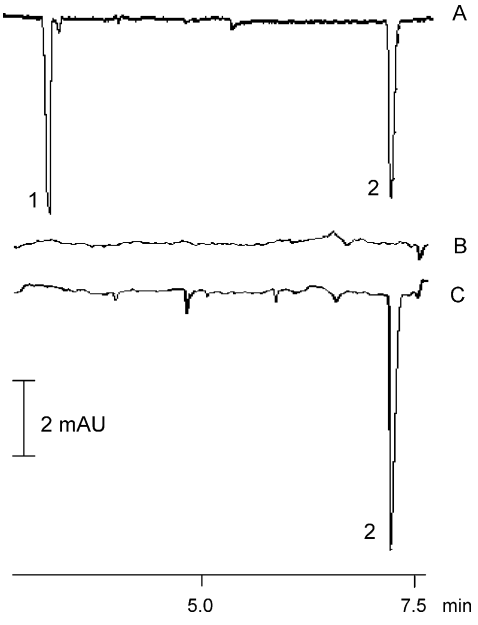

Fig. 7. Hydrolysis studies: analysis of carboxylic acids. (A) Standard solution containing malonic acid (1) and acetic acid (2). Soy germ capsules extracts prepared at 80% MeOH for 2 h and submitted to solid-phase extraction in octadecylsilica cartridges (B) followed by hydrolysis at 80 °C for 30 min (C). Separation conditions: 10 mmol L^{-1} 3,5-dinitrobenzoic acid and 0.5 mmol L^{-1} CTAB (pH 3.55); injection, 0.5 psi, 3 s; applied voltage, −20 kV; 58.5 cm × 75 μm i.d. capillary; 25 °C; indirect detection, 254 nm.

Table 3
Method validation regarding precision

Isoflavone[a]	Intra-day precision[b]		Inter-day precision[c]
	Migration time (%CV)	Peak area (%CV)	Concentration (%CV)
1	0.20	1.6	5.4
2	0.18	1.4	5.3
3	0.21	1.2	6.2
4	0.23	1.7	3.9
5	0.25	1.7	3.5
6	0.29	1.6	3.4

[a] Numbering according to Fig. 1.
[b] $n = 6$.
[c] $n = 18$ (3 days; two preparations and triplicate injection at each day).

the following validation parameters were evaluated: selectivity, precision, accuracy, linearity, limit of detection and limit of quantification.

3.5.1. Selectivity

The method selectivity was established by the near baseline separation of the major isoflavones in the sample extract (electropherogram of Fig. 5).

3.5.2. Precision

Repeatability (intra-day precision) was established by six consecutive injections of a mixture of six isoflavone standards at 25 μg mL^{-1} each. Repeatability of migration time and peak areas were better than 0.29 and 1.7% CV, respectively (Table 3). Intermediate precision (inter-day precision) was established for the analysis of two preparations of a hydrolyzed sample extract (Laboratory A) at three different days with triplicate injection of each sample. The results ranged from 3.4 to 6.2% CV (Table 3).

Table 4
Method validation regarding accuracy: recovery test

Isoflavone[a]	Original concentration (μg mL^{-1})	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)
1	20.4	20.0	40.4	99.8
2	24.7	20.0	45.2	102.6
3	9.10	20.0	28.9	99.1
4	514	50.0	566	103.6
5	416	50.0	467	102.4
6	159	50.0	210	101.1

[a] Numbering according to Fig. 1.

Table 5
Method validation regarding linearity and limits of quantification (LOQ)

Isoflavone[a]	Slope[b]	Intercept	S.E.	r^2	LOQ (μg mL^{-1})
1	1.54	0.0854	0.107	0.99999	0.69
2	1.35	0.129	0.0929	0.99999	0.68
3	1.50	0.102	0.104	0.99999	0.69
4	0.877	−0.0381	0.0961	0.99998	1.1
5	0.801	−0.0165	0.0966	0.99997	1.2
6	1.08	−0.108	0.0722	0.99999	0.67

S.E., standard error estimate; r^2, coefficient of determination.
[a] Numbering according to Fig. 1.
[b] Concentration range: 1.6–50 μg mL^{-1}.

3.5.3. Accuracy

The method accuracy was established by recovery tests at two concentration levels, 20 and 50 μg mL^{-1} (Table 4). Recoveries in the range of 99.1–103.6% were obtained. Also, different amounts of soy germ samples (50–500 mg) were weighed and submitted to the extraction procedure (2.00 mL 80% MeOH, 2 h). All extracts were dissolved in 100 mmol L^{-1} STB and water in the proportion of 1:1:8 (v/v/v) and submitted to analysis. The sum of peak areas for all isoflavones determined in each sample extract was plotted against the weight of soy germ samples showing a linear relationship (intercept = 0.00; slope = 0.484; $r^2 = 0.9999$). The curve linearity indicates that the amount of extracted isoflavones does not depend on the ratio soy germ weight to solvent for the studied ratios, suggesting that the proposed extraction procedure might be considered exhaustive.

3.5.4. Linearity and LOQ

The analytical curves comprised six standard solutions of isoflavone mixtures in the 1.6–50 μg mL^{-1} range with triplicate injection at each level. A linear relationship between peak area and isoflavones concentration was obtained with acceptable coefficients of correlation (0.9999 or greater) and intercepts close to the origin. Limits of quantification (LOQ) were obtained from the analytical curve statistics (LOQ = 10S.E./s, where S.E. is the curve standard error estimate and *s* its slope). The curves statistics as well as the limits of quantification are presented in Table 5. LOQ varied from 0.67 to 1.2 μg mL^{-1}.

3.6. Sample analyses

The results of quantitation of isoflavones in pharmaceutical capsules from different laboratories are compiled in Table 6. For

Table 6
Analysis of total isoflavones in commercial formulations

Isoflavone[a]	Laboratory A (mg/capsule)		Laboratory B (mg/capsule)	Laboratory C (mg/capsule)	Laboratory D (mg/capsule)	
	Before hydrolysis	After hydrolysis	After hydrolysis	After hydrolysis	Batch 1 after hydrolysis	Batch 2 after hydrolysis
1	0.211 ± 0.009	0.203 ± 0.004	2.72 ± 0.20	3.48 ± 0.06	nd	nd
2	0.304 ± 0.013	0.273 ± 0.004	1.23 ± 0.02	0.77 ± 0.03	8.28 ± 0.24	6.92 ± 0.13
3	0.089 ± 0.006	0.084 ± 0.003	1.25 ± 0.08	0.73 ± 0.01	9.48 ± 0.33	5.91 ± 0.15
4	6.12 ± 0.13	6.48 ± 0.17	18.05 ± 0.18	20.31 ± 0.37	5.89 ± 0.21	9.23 ± 0.19
5	4.63 ± 0.11	5.50 ± 0.14	2.22 ± 0.09	2.88 ± 0.11	2.84 ± 0.16	4.51 ± 0.15
6	1.88 ± 0.06	2.10 ± 0.005	32.89 ± 0.34	32.64 ± 0.69	1.55 ± 0.09	1.79 ± 0.05
Total amount determined	13.23 ± 0.34	14.64 ± 0.33	58.36 ± 0.91	60.8 ± 1.3	28.0 ± 1.0	28.36 ± 0.67
Total amount declared		15	60	63	30	30
Purity (%)		97.6	97.3	96.5	93.3	94.5

nd, not detected.
[a] Numbering according to Fig. 1.

Laboratory A, the sample was analyzed before and after hydrolysis, indicating that only the amounts of the isoflavones 4–6 increased after hydrolysis. This quantitative result further corroborates the peak assignment indicated in Fig. 6, where peaks 7–9 were attributed to the malonyl derivatives of the isoflavones 4–6. Also in Table 6, the summation of all isoflavones after sample hydrolysis was contrasted with the total amount declared by the manufacturer indicating good agreement (purity above 93%).

4. Conclusions

A fast and reliable method for the extraction and determination of isoflavones in soy germ capsules has been developed and validated using capillary electrophoresis in micellar medium. The electrolyte optimization was assisted by a 3^2 factorial design and a novel response function based on mobility and conductance was proposed. The presence of three unknown peaks in the electropherogram of the sample extract was attributed tentatively to malonyl isoflavone derivatives. This assignment was supported by migration behavior, UV spectra comparison and the analysis of the hydrolysis products. The method analytical performance makes it suitable for implementation in pharmaceutical laboratories for the routine analysis of isoflavone formulations.

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