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MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC) OF SECOISOLARICIRESINOL DIGLUCOSIDE – A SHORT REPORT

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Key words: micellar electrokinetic chromatography (MEKC), flaxseed, phenolics, lignans, secoisolariciresinol diglucoside (SDG)

Phenolic compounds were extracted from flaxseed using 80% methanol. Then the crude extract was hydrolysed at basic condition. SDG was separated from so prepared extract using a semi-preparative HPLC. Chemical structure of SDG was confirmed by ESI-MS. Micellar electrokinetic chromatography (MEKC) was used for separation of phenolics from the extract after hydrolysis. MEKC was found to offer a quicker analysis with high separation efficiency than the conventional HPLC method. Retention time for SDG was noted at 12.25 min (sample) and 11.95 (standard).

INTRODUCTION

Secoisolariciresinol diglucoside (SDG) (Figure 1) has been reported as the main phenolic constituent of flaxseed (*Linum usitatissimum* L) [Meagher *et al.*, 1999]. SDG belongs to lignans and its content in defatted flaxseed reaches 1–3% [Johnsson *et al.*, 2000]. Secoisolariciresinol diglucoside exerts an estrogenic effect by acting as a precursor of "mammalians" lignans: enteroradiol and enterolactone [Axelson *et al.*, 1982]. A reduced breast cancer risk has been reported for subjects with high urine and plasma levels of enteroradiol and enterolactone [Boccardo *et al.*, 2004]. SDG may also protect against prostate and colon cancer [Jenab & Thompson, 1996; Adlercreutz, 2002; Lin *et al.*, 2002]. Antioxidant activity of SDG was reported by Niemeyer and Metzler [2003]. The content of ligans in flaxseed made seeds of this plant an interesting raw material for food application within the concept of functional foods [Oomah & Mazza, 1998].

Micellar electrokinetic chromatography (MEKC), a hybrid of electrophoresis and chromatography, is the only electrophoretic technique that can be used for the separation of neutral analytes as well as charged ones. For neutral species, it is the partitioning in and out of the hydrophobic interior of the micelles that affects the analytes' retention in the capillary. This separation process, governed by polarity, is completely analogous to HPLC; however within solution, the stationary phase is actually moving [Strein *et al.,* 1999].

The present study reports on a facile MEKC method for the separation of SDG and other phenolic components from crude and hydrolysed extracts of flaxseed.

MATERIAL AND METHODS

Methanol, petroleum ether, NaOH, HCl, disodium tetraborate, Na_2HPO_4 of pure for analysis grade were acquired from the P.O.Ch. (Gliwice, Poland). Methanol, acetonitrile and acetic acid for HPLC analysis, cetyltrimethylammonium bromide, were obtained from Sigma Chemical Co. (St. Louis, MO).

Flaxseeds were obtained from a local drug store and used in this study. Seeds were ground in a commercial coffee mill and then defatted with petroleum ether in Soxhlet apparatus for 6 h. Phenolic constituents were extracted from the defatted meal with 80% (v/v) methanol at a material-to-solvent ratio of 1:10 (w/v) at 50°C for 30 min [Amarowicz *et al.*, 1995]. Extraction was carried out in dark-coloured flasks using a temperature controlled shaking water bath. The extraction process was repeated twice; supernatants were combined and solvent was removed under vacuum at 40°C on rotary evaporator. The prepared extract was stored at 4°C until further investigated.

SDG is present in flaxseed extract in a tan polymeric powder [Kamal-Eldin *et al.*, 2001; Johnsson *et al.*, 2002] and before analysis the process of hydrolysis must be carried out. In this study, the extract was subjected to base hydrolysis (1 g of extract suspended in 20 mL of 0.3 mol/L NaOH) for 2 days at room temperature followed by acidification to pH 3 using 2 mol/L aqueous HCl [Johnson *et al.*, 2000]. The hydrolysed extract was applied on the chromatographic column (14 × 2 cm) packed with RP-18 gel (40–63 μm, Merck, Darmstad, Germany). Sugars and salts were washed out with 200 mL of distilled water, phenolics were eluted by

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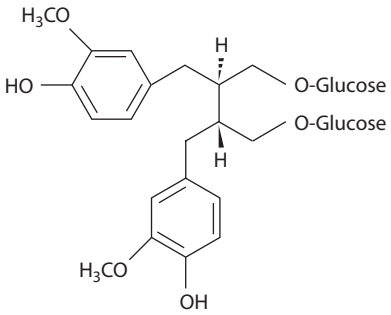

FIGURE 1. Chemical structure of secoisolariciresinol diglucoside (SDG).

200 mL of methanol and organic solvent was evaporated using a rotary evaporator.

Pure SDG was separated from the above-obtained concentrate using a semi-preparative HPLC method. A Shimadzu chromatographic system (Kyoto, Japan) consisting of a LC-10A pump, SPD-M10A UV-VIS diode array detector, SCL-10A system controller, semi-preparative LUNA C_{18} column (250 × 10 mm, 5 μ; Phenomenex, Torrance, CA, USA) was used; mobile phase comprising water-acetonitrile-acetic acid (88:10:2; v/v/v) [Amarowicz & Weidner, 2001]; flow rate 4 mL/min; and injection volume 500 μL; sample aw dissolved in methanol and detector was set at 280 nm.

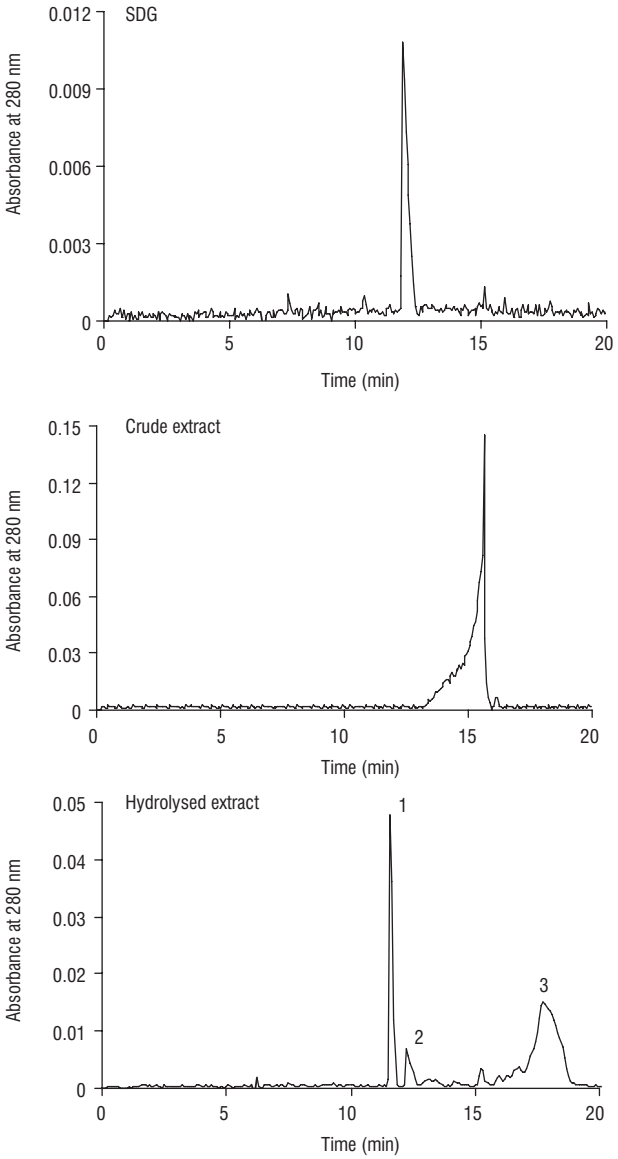

FIGURE 2. MEKC chromatogram of pure SDG, crude flaxseed extract and crude hydrolysed extract.

Electron Spray Ionization mass spectra (in negative mode) of SDG dissolved in 80% methanol (v/v) were obtained using a Shimadzu liquid chromatograph mass spectrometer LC MS – QP 8000 (Kyoto, Japan). Condition of analysis were: CDL temperature – 240°C; CDL voltage – 50 V; probe voltage – 3.5 kV, defragmentation voltage – 45 V; nebulizer gas flow – 2.8 mL/min, flow rate – 0.2 mL/min; injection – 10 μL.

Micellar electrokinetic chromatography of the pure obtained here SDG, crude extract, and crude extract after hydrolysis was performed using a Beckman 5510 capillary electrophoresis instrument with UV diode array detection. The buffer employed for MEKC was 18 mmol/L disodium tetraborate, 30 mmol/L Na_2HPO_4, 50 mmol/L cetyltrimethylammonium bromide (pH 7). An uncoated fused-silica capillary (50 μm *i.d.* × 67/60 cm) was used to separate the sample. Separation was carried out at 20 kV. The photodiode detector was set at 280 nm.

RESULTS AND DICUSSION

The ESI mass spectrum was characterized by the main ion with *m/z* of 685. This ion assigned to $[M\text{-}H]^-$ confirms the fact that the purified compound was secoisolariciresinol diglucoside. The MEKC chromatogram (Figure 2) shows the high purity of SDG standard obtained here from the hydrolysed extract by semi-preparative HPLC. The retention time of SDG was recorded at 11.95 min; lignan's UV spectrum showed the maximum at 290 nm (Figure 3).

The chromatogram of the crude flaxseed extract (Figure 2) showed only one broad peak with retention time at 15.63 min. The UV spectrum of the complex was characterized by maximum at 300 nm (Figure 3).

The well resolved peaks 1, 2, and 3 with retention times of 11.58, 12.25, and 17,92 min were recorded when the hydrolysed extract was analysed (Figure 2). The maxima of UV-DAD spectra assigned to peaks 1–3 were noted at 310, 290, 310, and 350 nm, respectively (Figure 3). Retention time of the peak 2 and UV spectrum support conclusion that this peak was assigned to secoisolariciresinol diglucoside.

Retention time of SDG obtained under MEKC conditions used was relatively short. Johnsson *et al.* [2000] obtained a baseline RP-HPLC separation in a gradient system with retention time of 19.5 min. When the RP-HPLC method was applied in an isocratic system (mobile phase of acetonitrile-water-acetic acid; 10:88:2; v/v/v), the retention time of SDG was noted at 50 min [Amarowicz *et al.,* 2004].

CONCLUSIONS

The retention time required to separate SDG from a hydrolysed flaxseed extract by MEKC with UV was shorter than in the case of analytical RP-HPLC. Micellar electrokinetic chromatography was found to offer a quicker analysis and less sample handling than the conventional HPLC method.

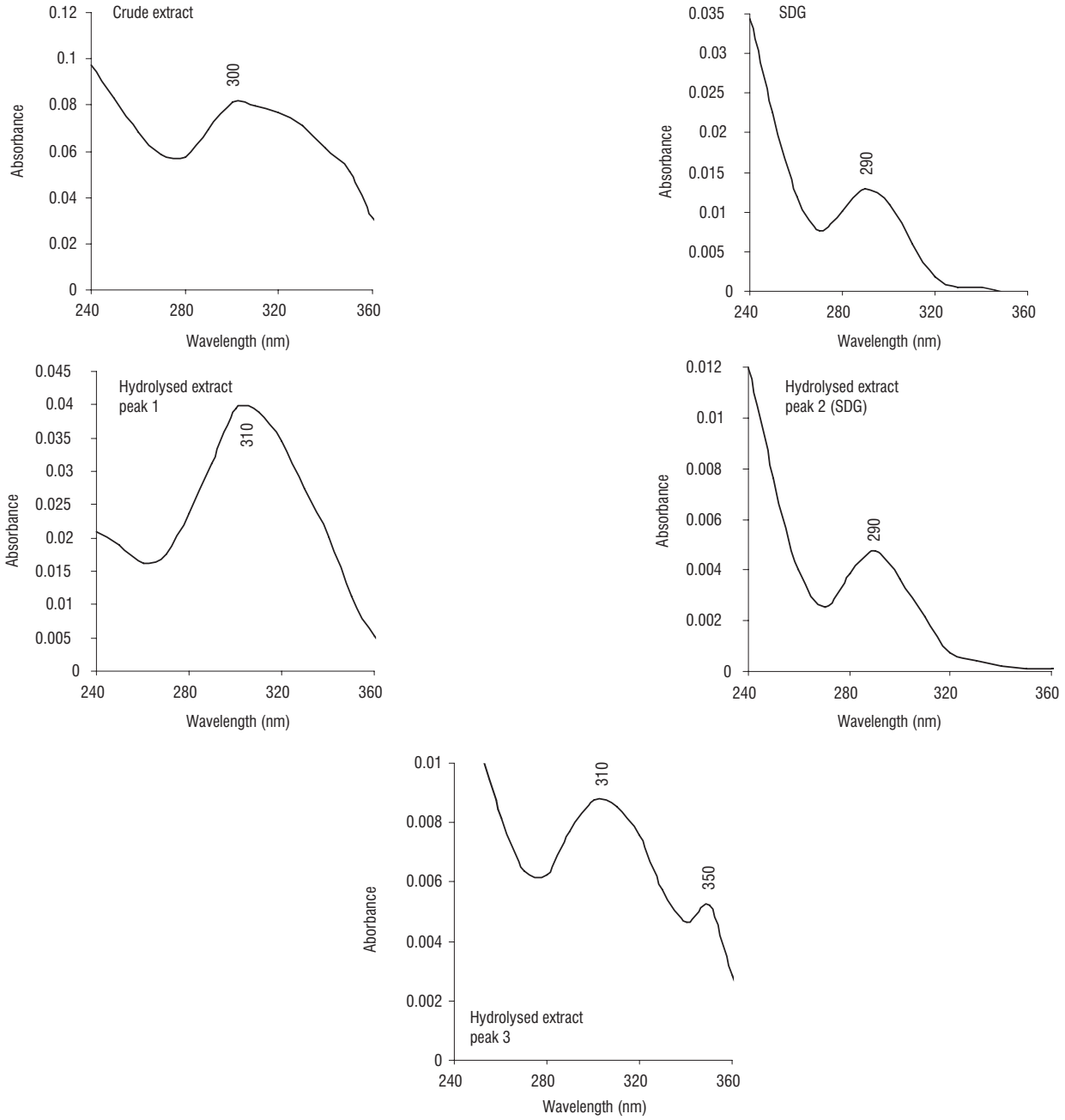

FIGURE 3. UV spectra of SDG, crude extract, and compounds 1-3 separated from the hydrolysed extract using MEKC method.

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MICELARNA ELEKTROKINETYCZNA CHROMATOGRAFIA (MEKC) DIGLUKOZYDU SEKOIZOLARICIREZINOLU (SDG) – KRÓTKIE DONIESIENIE

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Związki fenolowe wyekstrahowano z nasion lnu za pomocą 80% metanolu. Z surowego ekstraktu po zasadowej hydrolizie wyodrębniono diglukozyd sekoizolaricirezinolu (SDG) stosując semipreparatywną HPLC. Strukturę SDG potwierdzono za pomocą ESI-MS. Do rozdziału związków fenolowych w hydrolizowanym ekstrakcie użyto micelarnej elektrokinetycznej chromatografii (MEKC). Metoda ta w porównaniu z HPLC charakteryzowała się krótszym czasem analizy i wysoką zdolnością rozdzielczą. Czas retencji dla SDG wynosił 12.25 min (próba) i 11.95 (wzorzec).