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HPLC method with fluorescence detection for the quantitative determination of flaxseed lignans

Jatinder Kaur Mukker[1], Valeriya Kotlyarova[1], Ravi Shankar Prasad Singh, Jane Alcorn*

Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, Thorvaldson Building, 110 Science Place, University of Saskatchewan, Saskatoon, Saskatchewan S7N5C9, Canada

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ABSTRACT

We report a rapid and simple HPLC method with fluorescence detection for the quantification of the major flaxseed lignan, secoisolarisiresinol diglucoside (SDG) and its major metabolites. The method is specific for SDG, secoisolarisiresinol (SECO), enterodiol (ED) and entrolactone (EL) in rat serum. The assay procedure involves chromatographic separation using a Waters Symmetry C_{18} reversed-phase column (4.6 mm × 150 mm, 5 μm) and mobile phase gradient conditions consisting of acetonitrile (0.1% formic acid) and water (0.1% formic acid). SDG extraction from serum requires the use of Centrifuge filters while SECO, ED and EL are extracted with diethyl ether. The organic layer is evaporated and reconstituted in 100 μL of mobile phase and 50 μL of reconstituted sample or filtrate is injected onto the column. Total run time is 25 min. Calibration curves are linear ($r^2 \geq 0.997$) from 0.05 to 10 μg/mL for SDG and EL and 0.01–10 μg/mL for SECO and ED. Precision and accuracy are within USFDA specified limits. The stability of all lignans is established in auto-injector, bench-top, freeze–thaw and long-term stability at −80 °C for 30 days. The method's reasonable sensitivity and reliance on more widely available HPLC technology should allow for its straightforward application to pharmacokinetic evaluations of lignans in animal model systems such as the rat.

1. Introduction

Lignans are a class of diphenolic compounds widely distributed in the plant kingdom [1,2]. Flaxseed is one of the richest sources of lignans with secoisolarisiresinol diglucoside (SDG) (Fig. 1A) as the principal lignan form. Flaxseed also contains minor amounts of the aglycone form, secoisolariciresinol (SECO) (Fig. 1B), and other lignans such as metaresinol and larisiresinol [3,4]. In the mammalian gastrointestinal tract SDG is hydrolysed to its aglycone, SECO, possibly through β-glucosidase and β-glucuronidase activity [5,6]. SECO is further converted to the mammalian lignans, enterodiol (ED) (Fig. 1C) and enterolactone (EL) (Fig. 1D) in the presence of colonic gut microflora [1,7,8].

At present, research principally attributes the health effects of the flaxseed lignans to their antioxidant activity [9,10] estrogenic activity [11,12], or to their role as the principle precursor lignans to the mammalian lignans, ED and EL [13]. Uncertainty exists as to whether the plant lignans (i.e. SDG or SECO) and/or the mammalian lignans (i.e. EL and ED) mediate the putative health benefits associated with flaxseed lignan consumption [14–16]. A complete pharmacokinetic characterization of flaxseed lignans would contribute vital information on lignan effects *in vivo*. As flaxseed lignans receive increasing attention in the treatment of cardiovascular disease and cancer [17], their promotion will require an improved understanding of lignan absorption and disposition characteristics. Such a pharmacokinetic evaluation will require a simple and rapid analytical method for the quantitative determination of lignans in biological matrices.

We found only a few analytical methods for the quantitation of lignans in the mammalian system. These methods involved the use of HPLC with fluoroimmunoassay [18], gas chromatography (GC) [19], UV detection or liquid chromatography-mass spectrophotometry (LCMS) techniques [20–25]. LCMS is not universally available and the reported HPLC methods suffer from various disadvantages including lack of analytical sensitivity and failure to report procedures for the simultaneous determination of all major lignan metabolites of SDG. To the best of our knowledge, the use of fluorescence detection for the quantification of flaxseed lignans in biological matrices has not been demonstrated. Fluorescence detection often improves analytical sensitivity as compared to UV detection methods. Therefore, we developed a HPLC fluorescence detection method for the determination of SDG, SECO, ED and EL in a rodent model system (the rat) commonly used for preliminary pharmacokinetic evaluations of new chemical entities and bioactive components of plants.

* Corresponding author. Tel.: +1 306 966 6365; fax: +1 306 966 6377.
E-mail address: jane.alcorn@usask.ca (J. Alcorn).
[1] Authors having equal contribution.

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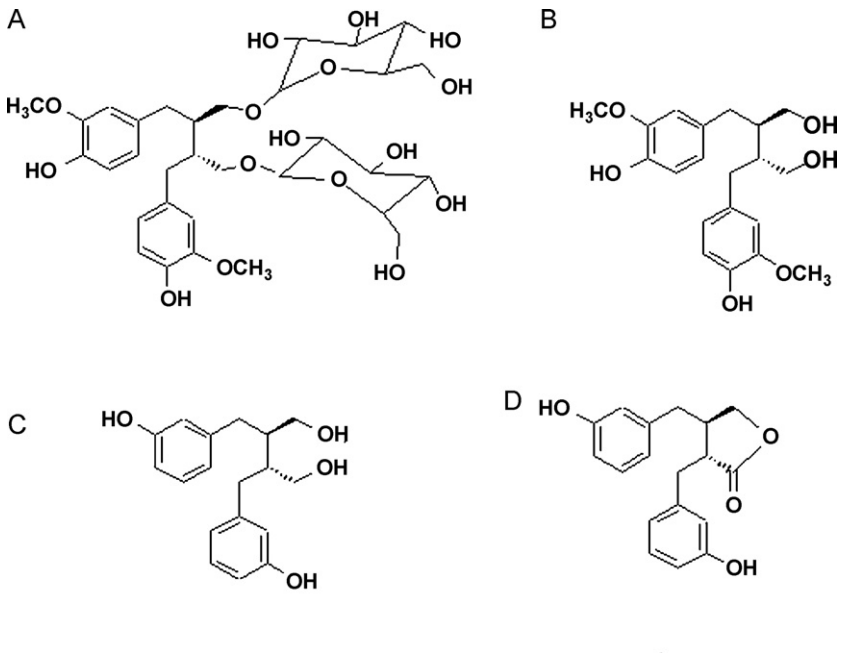

Fig. 1. Structural representation of Secoisolarisiresinol diglucoside (SDG) (A), Secoisolarisiresinol (SECO) (B), Enterodiol (ED) (C), and Enterolactone (EL) (D).

2. Materials and methods

2.1. Chemicals and reagents

SDG and SECO (>95% purity) were kind gifts from Agriculture and Agri-Food Canada, (Dr. Alister Muir). ED and EL were purchased from Sigma-Aldrich Canada Ltd (Oakville, ON). Umbelliferone (7-Hydroxycoumarin) and riboflavin were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). HPLC grade acetonitrile was purchased from Fisher Scientific Canada (Ottawa, ON). Diethyl ether was purchased from EMD Chemicals Limited (Gibbstown, NJ). Methanol was purchased from Caledon Laboratories (Georgetown, ON). A MilliQ Synthesis (Millipore, Bedford, MA) Water Purification system provided purified deionized water. All other chemicals used were analytical grade.

2.2. Instrumentation and chromatographic conditions

The HPLC (Agilent Technologies, Mississauga, ON) system consisted of a Series 1200 quaternary pump (G1311A) with online degasser (G1322A), autosampler (G1329A), and fluorescence detector (G1321A). Processed samples (50 μL) were injected onto a Waters Symmetry C_{18} column (4.6 mm × 150 mm, 5 μm). The analytes were eluted under gradient mode with mobile phase consisting of water with 0.1% formic acid (component A) and acetonitrile with 0.1% formic acid (component B) in different ratios delivered at a flow rate of 1 mL/min. Excitation wavelength was set at 277 nm and emission wavelength at 617 nm. The mobile phase was filtered through a 0.22 μm Nylon filter (Pall Scientific, Mississauga, ON) and degassed in an ultrasonic bath for 30 min prior to use. The column was maintained at room temperature (22 °C) and washed with water:methanol (50:50) after every use. The potential for autosampler carry over was reduced by injection of blank mobile phase after the highest calibration curve concentration. For SDG the mobile phase gradient conditions consisted of an initial isocratic condition of 85:15 component A:component B from 0 to 12 min, an increasing gradient from 15% to 50% of component B from 12 to 14 min and then 50–90% from 14 to 16 min, a decreasing gradient from 90% to 15% component B from 16 to 23 min, and a return to 85:15 component A:component B between 23 and 25 min. For SECO, ED and EL the gradient consisted of 85:15 component A:component B from 0 to 12, an increasing gradient from 15% to 50% component B and from 50% to 90% between 12 and 14 min and then 90% between 14 and 18 min, with a decreasing gradient from 90% to 15% component B from 18 to 20 min and a return to 85:15 component A:component B between 20 and 25 min.

2.3. Preparation of stock and working standard solutions

Stock solutions (1 mg/mL) of the lignans and internal standards (umbelliferone and riboflavin) were prepared by initial dissolution in methanol followed by dilution with mobile phase (70% component A:30% component B for SECO, ED, EL and umbelliferone; 80% component A:20% component B for SDG and riboflavin). Working solutions of the lignans (0.1–100 μg/mL) were prepared by serial dilution of the stock solution with mobile phase while working solutions of the internal standard were prepared by a single dilution of the stock solution to a concentration of 100 μg/mL. Quality control samples were prepared in a similar manner by a different analyst to achieve working stock solutions (as per USFDA guidelines) at the low quality control (LQC) (3-fold the lower limit of quantification (LLOQ)), middle quality control (MQC) and high quality control (HQC) (80% of the upper limit of quantification). These stocks were stored at −20 ± 5 °C, and used to prepare standard curve samples on the day of analysis. The stock solutions were stable up to 30 days (data not shown).

2.4. Preparation of calibration curve samples and quality control (QC) samples

Calibration curve samples were prepared on each day of analysis by adding 10 μL of individual working solutions to 90 μL of pooled rat blank serum (see Section 2.7) with vortex-mixing for 30 s. For the quality control samples a separate analyst added the appropriate volume of working solutions into pooled rat serum in bulk on the first analysis day and these samples were then individually aliquoted into polypropylene microcentrifuge tubes (disposable conical economy micro tubes with snap caps, 2 mL volume, Catalog

No. 14231-064, VWR Mississauga, ON) and stored at −80 ± 5 °C in the dark until analysis.

2.5. Sample preparation

For SECO, ED and EL, 10 μL of umbelliferone (internal standard) solution (100 μg/mL) was added to 100 μL of calibration standards, QC samples, or rat serum samples and vortex-mixed for 10 s. To all samples, 4 mL of diethyl ether was added, vortex-mixed for 10 min, and centrifuged at 4 °C at 780 × *g* in an Eppendorf microcentrifuge (Model 5804A, Brinkmann Instruments, Westbury, NY). The aqueous layer was snap frozen using liquid nitrogen and the organic layer was transferred to glass tubes and evaporated to dryness under vacuum at 40 °C in an evaporator (Centrivap Concentrator, Labconco Corporation, Kansas, MO). The residue was reconstituted in 100 μL of mobile phase, vortex-mixed for 2 min, transferred to HPLC vials and 50 μL was injected onto the column.

For SDG, 10 μL of riboflavin (internal standard) solution (25 μg/mL) was added to 100 μL of calibration standards, QC samples, or rat serum samples and briefly vortex-mixed. Samples were transferred to centrifuge filters (Modified PES 10K, 500 μL, VWR International, Mississauga, ON) and centrifuged at 13,300 × *g* in a microcentrifuge (Accuspin Micro17 centrifuge, Fisher Scientific Canada, Ottawa, ON) for 30 min. The filtrate was transferred to HPLC vials and 50 μL of sample was injected onto the column.

Recovery was determined at LQC, MQC and HQC. The peak areas of post-extracted or post-filtered serum samples were compared with those obtained from unextracted LQC, MQC and HQC samples prepared in mobile phase.

2.6. Validation procedures

A complete validation for the assay of SDG, SECO, ED and EL in rat serum was performed in accordance with USFDA guidelines [26]. Specificity was assessed by analysis of serum from six different rats to determine the absence of endogenous substances with similar retention times to the lignans and internal standards.

The limit of detection (LOD) was the lowest detectable concentration with a signal-to-noise ratio of 3. Lowest Limit of quantification (LLOQ) was determined at the lowest concentration that gave precision and accuracy values within 20% of the mean and nominal values, respectively. The LOQ was the lowest concentration on the calibration curve and the linearity from LLOQ to 10,000 ng/mL was assessed by processing a 10-point calibration curve on several different days. The ratio of peak areas of the analytes and internal standards were plotted against the nominal concentrations of the calibration curve samples. A linear least-squares regression analysis, using $1/X^2$ as weighting factor, was conducted to determine slope, intercept and coefficient of determination (r^2) to demonstrate linearity of the method. Calculated concentrations of the calibration curve samples had no more than ±15% deviation from nominal concentration, except at LLOQ which was less than ±20%.

The intra- and inter-day precision and accuracy of the method was determined by analyzing six replicates at each of LLOQ, LQC, MQC, and HQC on three different days. Precision was expressed as % relative standard deviation (RSD) with acceptance criteria of RSD ±15% at each concentration except at LLOQ, which was allowed RSD ±20%. Accuracy (percent) was expressed as [(calculated amount/predicted amount) × 100] with acceptance set at ±15% of the nominal concentrations of QC samples except at LLOQ, where it was set at ±20%.

Stability studies included freeze/thaw stability, bench top stability, and long-term stability, which were performed at LQC, MQC, and HQC, and autosampler stability was tested at these concentrations but also included LLOQ. Freeze/thaw stability was tested after three freeze/thaw cycles spaced at least 24 h apart with sample storage at −80 °C between each thaw. Bench top stability was established at room temperature for SDG and on ice for SECO, ED and EL for 6 h. To determine autosampler stability, processed samples were maintained in the autosampler for at least 24 h prior to injection. Predicted concentrations were calculated using fresh calibration curve standards. Long-term stability was assessed after 30 days of storage at −80 ± 5 °C.

2.7. Application to a pharmacokinetic study in rat

An *in vivo* intravenous pharmacokinetic study was performed in male Wistar rats ($N = 4$, weight range 250–300 g and age range 7–9 weeks) obtained from the Animal Resources Centre (ARC), University of Saskatchewan, Canada to demonstrate the applicability of the validated bioanalytical method. SECO was administered intravenously at a dose of 20 mg/kg in a parenteral formulation consisting of PEG 300, Tween 80, benzyl alcohol, and ethanol in a 65:8:3:24 (v/v) mixture. Femoral and jugular veins were surgically cannulated under isoflurane anaesthesia for administration of SECO and for blood sampling, respectively, 24 h prior to SECO dosing. Blood samples (250 μL) were collected into polypropylene microcentrifuge tubes (disposable conical economy micro tubes with snap caps, 2 mL volume, Catalog No. 14231-064, VWR Mississauga, ON) at 0–12 h post-dosing. Blood was allowed to clot at room temperature for 30 min and serum was collected following centrifugation at 5000 × *g* for 10 min at 4 °C and stored frozen at −80 ± 5 °C until analysis. Rat serum (100 μL) samples were spiked with umbelliferone and processed as described above, and the analysis run was accepted based on the performance of QC samples. The criteria for acceptance of the analytical runs encompassed the following: (i) not more than 33% of the QC samples were greater than ±15% of the nominal concentration (ii) not less than 50% at each QC concentration level must meet the acceptance criteria. The animal protocol for the pharmacokinetic analysis of SECO was conducted in accordance with Canadian Council of Animal Care (CCAC) guidelines and approved by University Committee on Animal Care and Supply at the University of Saskatchewan.

2.8. Data and statistical analysis

A Student's *t*-test was used to determine whether slopes and intercepts of the calibration curves were significantly different from zero using Prism 4.0 (GraphPad Prism, San Diego, CA, USA). The level of significance was set at $P < 0.05$. Noncompartmental pharmacokinetic (PK) analysis for SECO was performed using WinNonLin 4.1 (Pharsight Inc., Mountain View, CA).

3. Results and discussion

This HPLC method with fluorescence detection represents a relatively simple and rapid bioanalytical technique for the major plant lignan, SDG, and its major metabolites SECO, ED and EL, using a widely available pharmaceutical analysis technology. Although the advantage of analytical sensitivity afforded by LC–MS analysis is indisputable, its lack of widespread availability limits pertinent evaluations of lignan pharmacology in mammalian systems. Our relatively straightforward and reasonably sensitive HPLC method for the analysis of lignans derived from flaxseed offers significant advantages in terms of sensitivity and ease of selectivity, sample preparation, and lower volume of sample requirements as compared to previously reported HPLC methods [6,20–23], which should allow for enhanced investigations into lignan pharmacology.

Many of the reported methods describe quantification of flaxseed lignans from flaxseed [20,21,23,24,27,28] or flax products

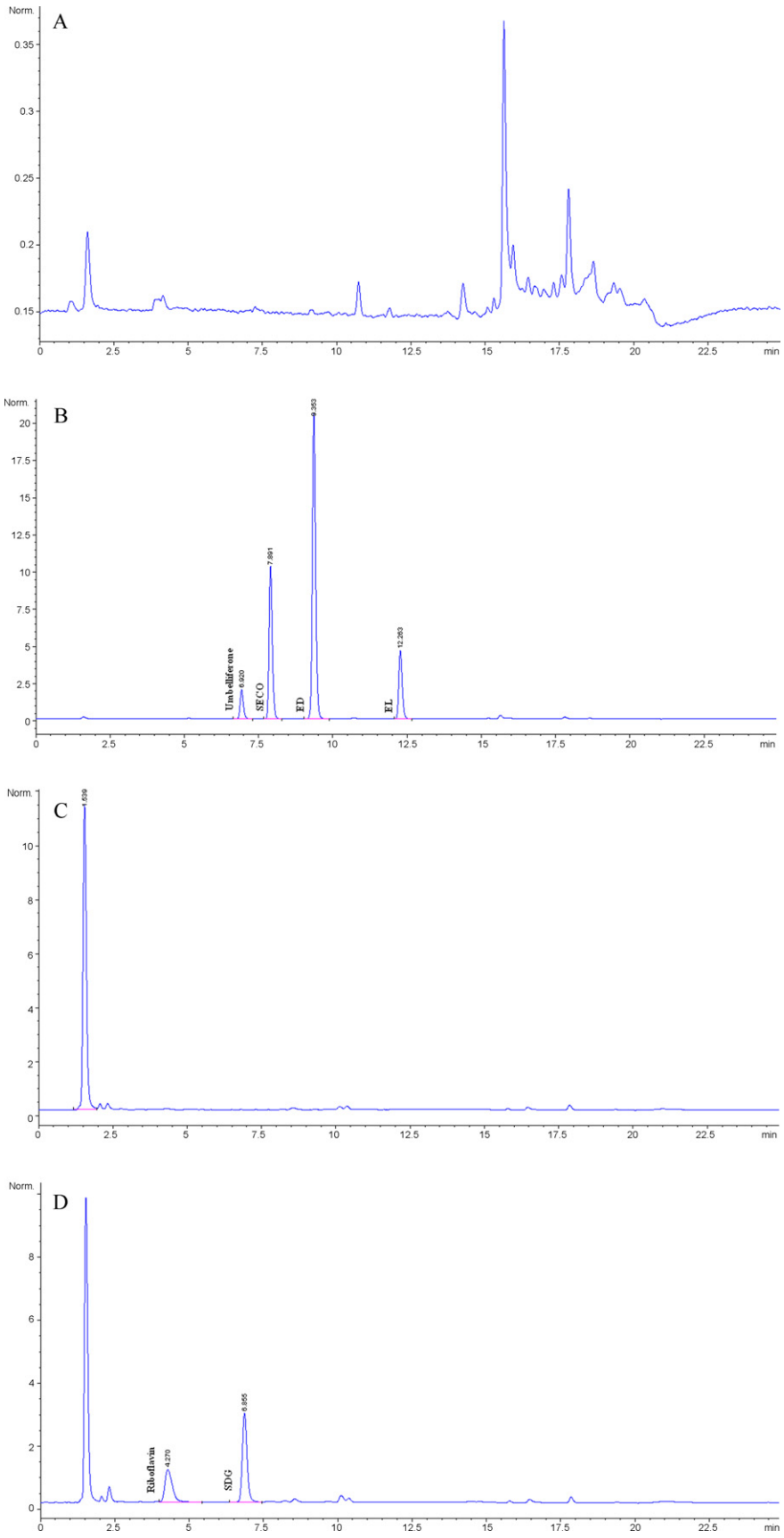

Fig. 2. Representative HPLC chromatograms of rat blank serum for SECO (10 μg/mL), ED (10 μg/mL) and EL (10 μg/mL) (A), rat serum spiked with Umbelliferone (10 μg/mL), SECO, ED and EL (B), rat blank serum for SDG (C), and rat serum spiked with Riboflavin (2.5 μg/mL) (IS) and SDG (10 μg/mL) (D).

Table 1
Intraday assay precision and accuracy for SDG, SECO, ED and EL in rat serum ($N = 6$).

QC levels*	Precision[a]				Accuracy[b]			
	SDG	SECO	ED	EL	SDG	SECO	ED	EL
LLOQ								
(Day-1)	6.5	8.0	11.6	7.4	91.1	96.8	87.4	94.1
(Day-2)	3.5	9.6	5.7	5.8	86.2	91.5	84.9	93.6
(Day-3)	13.3	4.5	6.1	7.4	101.4	99.9	99.6	89.9
LQC								
(Day-1)	2.4	5.6	2.0	2.9	96.8	104.3	89.0	100.0
(Day-2)	3.7	5.3	3.2	5.3	90.2	95.8	89.8	101.8
(Day-3)	4.6	8.6	4.4	5.7	100.9	100.6	97.3	89.5
MQC								
(Day-1)	2.5	4.2	3.9	4.2	100.0	107.2	101.3	105.0
(Day-2)	3.6	4.5	3.6	3.9	98.4	93.3	100.7	102.8
(Day-3)	5.1	5.5	5.3	4.4	98.7	95.8	104.2	89.5
HQC								
(Day-1)	3.4	1.4	1.3	1.1	100.5	104.3	89.9	94.8
(Day-2)	4.8	1.6	1.6	1.3	104.2	90.8	90.4	97.2
(Day-3)	3.9	4.0	4.6	4.7	98.8	103.9	103.2	88.5

[a] Expressed as % RSD ((SD/mean) × 100%).
[b] Calculated as (mean determined concentration/nominal concentration) × 100%.
* LLOQ for SDG and EL 50 ng/mL and for SECO and ED is 10 ng/mL; LQC for SDG and EL is 150 ng/mL and for SECO and ED is 30 ng/mL; MQC is 4000 ng/mL and HQC is 8000 ng/mL for all four lignans.

[28–30]. A fewer number report methods for lignan quantification in human or rat biological matrices [22,31–33]. Some of these methods use the less widely available LC–MS technology for quantification of ED and EL [22,33]. These methods do not describe the quantification of SDG or SECO, thereby limiting their application for assessments of the major lignan form of flaxseed (SDG) and its relevant metabolites (SECO, EL, ED). Nurmi et al. achieved very sensitive detection limits for the quantification of secoisolariciresinol, enterodiol and enterolactone in human plasma using coulometric electrode array detection, but the accuracy of the method was only 69% for ED and SDG was not included in the analysis [32]. Gamache et al. used HPLC with coulometric assay to quantify ED and EL in rat plasma and human urine with a low LOD, but SDG and SECO was not included in the analysis [31]. In general, the reported methods for quantification of lignans in biological matrices fail to allow the simultaneous estimation of all major metabolites of SDG produced *in vivo*. Our current method simultaneously quantifies all major metabolites of SDG in rat serum using low sample volumes (0.1 mL), which is necessary to permit serial blood sampling and the pharmacokinetic characterization of lignans in rat. Our method also quantifies SDG using slightly different extraction methods and mobile phase conditions.

Given the widely divergent physicochemical properties of the lignans, the use of two different extraction procedures and elution conditions was inevitable. With the two glucose moieties, SDG is a highly polar compound compared to its aglycone form, SECO, and the mammalian lignans, ED and EL. Although we attempted to establish a consistent mobile phase gradient condition and extraction method suitable for all relevant lignans derived from flaxseed, unacceptable run times, interfering endogenous peaks and poor extraction efficiencies thwarted these efforts. Hence, SDG requires analysis separate from SECO, ED and EL. Despite this disadvantage, though, our method does offer a suitably sensitive analytical alternative to LC–MS methods.

3.1. Method validation

Fig. 2 presents representative HPLC chromatograms of lignans and their respective internal standards spiked into rat serum. The chromatograms demonstrate that the method is specific with the absence of endogenous peaks that co-elute with the lignans and internal standards. The chromatographic conditions used for the analysis gave retention times for riboflavin (internal standard) and SDG of 4.3 and 6.8 min, respectively, while retention times for umbelliferone (internal standard), SECO, ED, and EL were 7.0, 7.9, 9.4 and 12.3, respectively. Absolute recovery of SDG was 83.4 ± 10.7, 99.0 ± 1.9 and 95.3 ± 1.1% at LQC, MQC and HQC, respectively, and recovery for riboflavin at 2.5 μg/mL was 85.0 ± 3.0%. The absolute recovery of SECO was 87.7 ± 9.4, 91.6 ± 9.3 and 93.7 ± 3.3% at LQC, MQC and HQC, respectively. The recovery of ED was 91.2 ± 5.8, 95.6 ± 5.2 and 90.2 ± 7.0% at LQC, MQC and HQC, respectively, and the recovery of EL was 88.2 ± 12.8, 84.7 ± 5.8 and 80.2 ± 13.0% at LQC, MQC and HQC, respectively. The recovery of umbelliferone at 10 μg/mL from rat serum was 94.0 ± 6.0%.

The limit of detection (LOD) for SDG, SECO, ED and EL was 16.6, 3.3, 3.3 and 16.6 ng/mL, respectively, and the lowest limit of quantification (LLOQ) was 50, 10, 10, and 50 ng/mL, respectively. The method was linear over a concentration range of 10–10,000 ng/mL for SECO and ED, and 50–10,000 ng/mL for SDG and EL with coefficient of determination values greater than 0.997 for all calibration curves. The relevant slope values and coefficients were statistically different from zero ($p < 0.05$). The average percent accuracy across different standard concentration levels varied from 93.6 to 104.6 for SDG and 93.4–109.6 for SECO, ED and EL, while average percent coefficient of variation (CV) ranged from 1.21 to 3.93 for SDG and from 0.18 to 13.04 for SECO, ED and EL (data not shown).

Tables 1 and 2 summarize the intra- and interday precision and accuracy data. Overall intraday and interday precision evaluations gave CV values of less than 13.3% and accuracy was within 10% of the nominal values. This data suggest the method is both accurate and precise in rat serum.

The stability of lignans derived from flaxseed was established in rat serum under different storage conditions that included bench top (6 h) and autosampler (24 h) stability at room temperature, freeze/thaw (3 cycles) stability, and 30-day storage at −80 °C stability. These were assessed at three different quality control levels and our data suggest that the lignans were stable in the auto-injector for 24 h, up to three freeze–thaw cycles, on the bench top for 6 h at room temperature for SDG and on ice for SECO, ED and EL, and for 30 days with storage at −80 ± 5 °C. SECO, ED and EL were not stable on the bench top for 6 h.

3.2. SECO pharmacokinetics following intravenous bolus injection

To demonstrate applicability of this method, a pharmacokinetic analysis of SECO was performed following a bolus intravenous

Table 2
Interday assay precision and accuracy for SDG, SECO, ED and EL in rat serum ($N = 6$).

QC levels*	Precision[a]				Accuracy[b]			
	SDG	SECO	ED	EL	SDG	SECO	ED	EL
LLOQ	9.6	8.0	10.6	7.3	87.3	96.1	90.6	92.7
LQC	5.9	7.2	5.3	6.3	96.0	100.2	92.1	96.6
MQC	3.7	7.7	4.4	8.6	99.1	98.7	102.3	99.8
HQC	4.5	7.0	7.4	4.1	101.1	99.7	94.4	92.7

[a] Expressed as % RSD ((SD/mean) × 100%).
[b] Calculated as (mean determined concentration/nominal concentration) × 100%.
* LLOQ for SDG and EL 50 ng/mL and for SECO and ED is 10 ng/mL; LQC for SDG and EL is 150 ng/mL and for SECO and ED is 30 ng/mL; MQC is 4000 ng/mL and HQC is 8000 ng/mL for all four lignans.

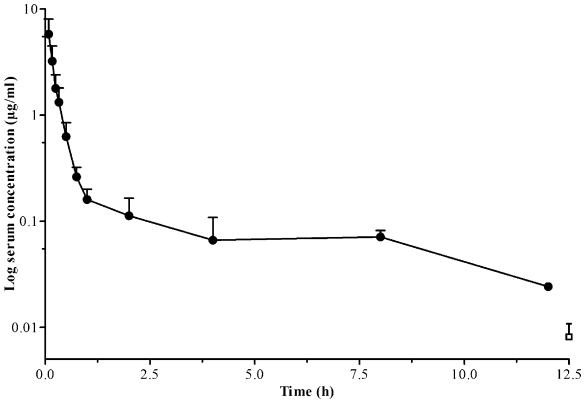

Fig. 3. Log mean ± SD serum concentration versus time profile of secoisolariciresinol (SECO) (●) and enterodiol (ED) (□) following an intravenous bolus administration of SECO (20 mg/kg) to male Wistar rats ($N = 4$).

injection. All QC samples met the acceptance criteria (data not shown). The mean serum SECO concentration versus time profile is shown in Fig. 3. Systemic clearance (Cl_s) for SECO was 8.0 ± 1.9 L/h kg, elimination rate constant (k) was 0.20 ± 0.10 h^{-1} and half life ($t_{1/2}$) was 4.1 ± 1.5 h. Volume of distribution (V_d) for SECO was 45.0 ± 11.7 L/kg and area under curve ($AUC_{0-\infty}$) was 2.6 ± 0.6 h μg/mL. The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the pharmacokinetics of SECO following an intravenous bolus injection. The single ED determination was likely due to enterohepatic recirculation as SECO becomes conjugated in the liver to glucuronide and sulfate metabolites, which are subsequently excreted into the gastrointestinal lumen [6]. Deconjugated and unabsorbed SECO, which becomes available in the colon, undergoes bacterial metabolism to the mammalian lignans, which are subsequently absorbed and also undergo extensive conjugation by the gastrointestinal mucosa and liver [6,34]. Treatment of serum samples with β-glucuronidase/sulfatase would likely result in the quantification of much higher levels of SECO, ED and EL as the glucuronic acid and sulfate moieties become removed making the parent form available for analysis. The highly polar nature of the glucuronic acid and sulfate conjugates would result in their rapid elution during chromatographic separation under the mobile phase conditions required for SECO, ED and EL analysis.

4. Conclusion

With the growing interest in flaxseed lignans and their association with a variety of health benefits, including prevention of cancer, cardiovascular diseases and hyperlipidemia, availability of a more widely accessible analytical method is necessary to improve our understanding of lignan pharmacology. The currently available HPLC methods tend to lack analytical sensitivity or accuracy, may require large sample volumes, or fail to report the simultaneous determination of all SDG metabolites, while the existing LC–MS analytical methods have more restricted accessibility to research laboratories. Our HPLC-fluorescence detection method is relatively simple and provides reasonable analytical sensitivity for the simultaneous quantification of the major metabolites of SDG, the principal lignan of flax. This method should be suitable for a complete pharmacokinetic analysis of lignans in suitable animal model species with possible extension to human clinical trial evaluations.

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