(10) Publication No: **IN202641059114 A1**
(22) Date of Filing: 09-05-2026
(43) Publication Date: 22-05-2026
Journal No: 21/2026

(19) Intellectual Property Office, India

(12) INDIAN PATENT APPLICATION

(54) Title: **Green extraction of sdg from flaxseed: development of a sustainable analytical method**

(51) International Classification:
G01N 30/02, G01N 21/3577, G01N 30/06, C07H 1/08 G01N 21/359

(21) Application No: **202641059114**

(31) Priority Document No: --

(32) Priority Date: --

(86) International Application No: --
Filing Date: --

(87) International Publication No: --

(61) Patent of Addition to Application No: --
Filing Date: --

(62) Divisional to Application No: --
Filing Date: --

(71) Name of Applicant(s):
Dr. Karthika Paul,

(72) Name of Inventor(s):
1. Ms. Anusha N
2. Ms. Narisetty Ahalya
3. Ms. Aathmika
4. Mr. Tushar Tiwari
5. Dr. Deepraj Paul
6. Dr. R S Chandan

(57) Abstract:

The present invention discloses a green and efficient method for extraction, isolation, characterization and quantitative analysis of secoisolariciresinol diglucoside (SDG) from flaxseed (Linum usitatissimum L.). The extraction is carried out using ethanol-water (50–70% v/v) and Tween 80 (1–5% v/v) followed by microwave-assisted extraction at 25–30°C for 30 minutes, water bath treatment, filtration and drying to obtain lignan-rich extract. Isolation is performed using 1,4-dioxane:ethanol (1:1 v/v), hydrolysis with methanolic NaOH (20mM, pH 8), acidification and column chromatography to obtain SDG with Rf value approximately 0.52. The characterization of SDG using Fourier Transform Infrared spectroscopy shows absorption bands ranging 3285–3326 cm^1, 1514–1649 cm^1, and 996–1153 cm^1, corresponding to hydroxyl, aromatic, and glycosidic functional groups, respectively. The ^1H Nuclear Magnetic Resonance spectroscopy shows signals in the range of 6.30–6.81 ppm, 4.39–5.20 ppm, 3.20–4.13 ppm, 2.80–3.50 ppm, and 1.90–2.70 ppm, confirming the presence of aromatic protons, anomeric protons, sugar moieties, and lignan backbone of SDG. The present invention provides an UFLC method for determination of SDG using BDS Hypersil C18 column with retention time approximately 4.1 minutes shows linearity (10–100 µg/mL, $r^2 = 0.9999$), precision (%RSD 2%), accuracy (98–102%), LOD 0.33 µg/mL and LOQ 1.00 µg/mL and stability-indicating capability under forced degradation conditions.

FORM 2

THE PATENTS ACT, 1970

(39 OF 1970)

&

THE PATENTS RULES, 2003

COMPLETE SPECIFICATION

(SECTION 10, RULE 13)

TITLE OF THE INVENTION

GREEN EXTRACTION OF SDG FROM FLAXSEED: DEVELOPMENT OF A SUSTAINABLE ANALYTICAL METHOD

APPLICANT

Name: Dr. Karthika Paul, **Nationality:** Indian, **Address:** Assistant Professor, Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru-570015, Karnataka, India

The following specification particularly describes the invention and the manner in which it is to be performed.

FIELD OF THE INVENTION

The present invention relates to extraction, isolation, characterization, and quantitative analysis of secoisolariciresinol diglucoside (SDG) from flaxseed using a green and efficient approach and development of a stability-indicating Ultra-Fast Liquid Chromatography (UFLC) method.

BACK GROUND OF THE INVENTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among females of reproductive age. The disease causes disbalanced inter-related hormonal system in hypothalamus, pituitary gland, and ovaries. PCOS may be resulted in infertility, metabolic syndrome, obesity, diabetes, cardiovascular risks, depression, sleep apnea, cancer, and liver disease. The metabolic signaling pathways play an important role in the development and progression of PCOS. Among these, the AMP-activated protein kinase (AMPK), sirtuin-1 (SIRT1), and glycolysis pathways are key regulators of cellular energy metabolism, glucose homeostasis, and inflammatory responses. Various medicines are mainly used to regulate menstrual cycles, help ovulation, and improve metabolic problems. The medicines can provide immediate relief to the condition but do not offer a permanent solution. However, long-term use of these medicines may cause side effects, may not always work effectively, and may not be well tolerated by patients. Therefore, there is growing interest in finding alternative treatments, especially from natural sources, that may be safer and more sustainable for managing PCOS.

Flaxseed (*Linum usitatissimum* L.) contains, Secoisolariciresinol diglucoside (SDG), a predominant lignan and is metabolized to enterodiol and enterolactone exhibiting antioxidant, anti-inflammatory, and estrogen-modulating activities.

Various methods are available to extract bioactive from flaxseed. However, conventional extraction methods are associated with limitations such as use of non-environmentally friendly solvents, longer extraction time, lower extraction efficiency, and possible degradation of bioactive compounds. Therefore, there exists a need for a simple, efficient, and environmentally friendly extraction

method for obtaining secoisolariciresinol diglucoside (SDG) from flaxseed with improved yield and stability.

OBJECTS OF THE INVENTION

The main object of the present invention is to provide a method for extraction of secoisolariciresinol diglucoside (SDG) from flaxseed using a surfactant-assisted green solvent system.

Another object of the invention is to provide isolation and characterization of secoisolariciresinol diglucoside (SDG).

Another object of the invention is to provide a green stability-indicating Ultra-Fast Liquid Chromatography (UFLC) method for quantitative determination of secoisolariciresinol diglucoside (SDG).

SUMMARY OF THE INVENTION

The present invention provides extraction of secoisolariciresinol diglucoside (SDG) from flaxseed using ethanol-water and Tween 80, microwave-assisted extraction, water bath treatment, drying, and recovery of lignan-rich extract.

In an embodiment, the present invention discloses that the isolated compound is characterized by FT-IR and ^{1}H NMR spectroscopy.

In an embodiment, the present invention provides an Ultra-Fast Liquid Chromatography (UFLC) method using ethanol and water mobile phase is developed and validated showing retention time of approximately 4.1 minutes, linearity with regression equation $y = 8130.7x - 8219.6$ and correlation coefficient (r^2) 0.9999, precision (%RSD $\leq$ 2%), accuracy (98–102%), LOD 0.33 µg/mL and LOQ 1.00 µg/mL.

Brief Description of drawings

In the drawings accompanying the specification, Figure 1 shows procedure followed for extraction of SDG from Flax seed.

In the drawings accompanying the specification, Figure 2 shows calibration curve, IR Report of Standard SDG and IR report of extract.

In the drawings accompanying the specification, Figure 3 shows NMR Report of Standard SDG, NMR Report of extract and Chromatogram of standard SDG and Chromatogram of extract.

In the drawings accompanying the specification, Figure 4 shows Calibration curve of SDG and Overlay graph of linearity concentrations.

In the drawings accompanying the specification, Figure 5 shows Chromatogram of Unstressed sample, Chromatogram of Photolytic degradation, Chromatogram of Acid degradation, Chromatogram of Base degradation, Chromatogram of Peroxide degradation and Chromatogram of Thermal degradation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for the extraction of lignans, particularly secoisolariciresinol Di glucoside (SDG), from flaxseed (*Linum usitatissimum* L.) using a surfactant-assisted green extraction approach.

1. Preparation of Flaxseed Material

Flaxseeds are initially defatted using conventional methods to obtain defatted flaxseed meal. The defatted meal is subsequently ground into a fine powder to increase surface area and facilitate efficient extraction of lignan compounds.

2. Preparation of Extraction Solvent

An extraction solvent is prepared comprising ethanol and water in a concentration range of approximately 50–70% (v/v) ethanol, combined with 1–5% (v/v) Tween 80 as a surfactant. The presence of the surfactant enhances the solubilization and release of lignan compounds from the plant matrix.

3. Solid–Solvent Mixing

The powdered defatted flaxseed meal is mixed with the prepared extraction solvent in a solid-to-solvent ratio of approximately 1:20 (w/v) to ensure adequate contact between the plant material and the extraction medium.

4. Microwave-Assisted Extraction

The mixture is subjected to microwave-assisted extraction at a controlled temperature of about 25–30°C for approximately 30 minutes. Microwave irradiation facilitates rapid penetration of the solvent into the plant matrix and enhances the release of bioactive constituents.

5. Water Bath Treatment

Following microwave treatment, the extract is further processed by water bath heating to promote improved dissolution and recovery of lignan compounds, particularly SDG.

6. Recovery of Crude Extract

The resulting mixture is allowed to cool and the crude extract is separated from the solid plant residue using suitable filtration or separation techniques.

7. Drying of Extract

The obtained crude extract is subsequently subjected to drying to remove residual solvent, resulting in an extract enriched with lignans, particularly secoisolariciresinol Di glucoside (SDG). This method provides an efficient and environmentally friendly approach for extracting SDG from flaxseed using a surfactant-assisted green solvent system combined with microwave-assisted extraction.

Column Chromatography and TLC Analysis of Flaxseed Extract:

The isolation of Secoisolariciresinol diglucoside (SDG) from flaxseed was initiated by preparing a lignan-rich extract from defatted flaxseed powder. Cleaned and dried seeds of *Linum usitatissimum* were finely powdered and subjected to defatting using hexane to remove lipid components. The defatted meal was subsequently extracted using a 1,4-dioxane:ethanol (1:1 v/v) solvent system under continuous stirring. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40–45°C to obtain a crude lignan extract. To release SDG from lignan oligomeric complexes, the extract was subjected to mild alkaline hydrolysis using methanolic NaOH (20 mM, pH 8) at approximately 50°C. The hydrolyzed mixture was then acidified to pH 3 using dilute sulfuric acid and concentrated to yield a semi-solid residue containing lignan constituents. The concentrated extract was purified by column chromatography using silica gel as the stationary phase. The sample was carefully loaded onto the column and eluted using solvent systems of gradually increasing polarity. Fractions were collected sequentially and monitored by thin layer chromatography (TLC). For TLC analysis, aliquots of the collected fractions were

spotted onto silica gel plates and developed using a chloroform:methanol (8:2 v/v) mobile phase. After development, the plates were visualized under UV light (365 nm), which revealed characteristic bands corresponding to lignan compounds. The retention factor (Rf) values were calculated using the following expression:

Rf = distance travelled by compound / distance travelled by solvent

Fractions exhibiting an Rf value of approximately 0.52, corresponding closely with the SDG reference standard, were pooled and concentrated to obtain purified SDG. The isolated compound was subsequently subjected to further spectroscopic and analytical characterization.

Attenuated Total Reflectance (ATR):

Fourier Transform Infrared (FT-IR) spectroscopy was employed for the identification and structural characterization of secoisolariciresinol diglucoside (SDG) isolated from flaxseed extract. The FT-IR spectra of the reference SDG standard and the isolated sample were recorded using a PerkinElmer Spectrum IR spectrophotometer equipped with an ATR accessory. The spectra were recorded in the mid-infrared region of 4000–500 cm^{-1} under identical instrumental conditions. A small quantity of the dried sample was placed directly onto the ATR crystal and scanned after performing background correction to eliminate atmospheric interference. The spectra were recorded at a spectral resolution of 4 cm^{-1} with 16 scans to obtain a clear and reproducible spectrum. The obtained spectra were expressed as percentage transmittance versus wavenumber. For identification and characterization, the FT-IR spectrum of the isolated extract was compared with that of the reference SDG standard. The presence of characteristic absorption bands corresponding to phenolic hydroxyl groups, aromatic skeletal vibrations, and glycosidic C–O stretching vibrations typical of lignan glycosides was used as the basis for structural identification. The similarity in the spectral patterns between the standard and the extracted sample confirmed the presence of secoisolariciresinol diglucoside (SDG) in the flaxseed extract.

NMR Analysis:

Nuclear Magnetic Resonance (NMR) spectroscopy was employed for the structural characterization and confirmation of secoisolariciresinol diglucoside

(SDG) isolated from flaxseed extract. The NMR spectra of the isolated compound were recorded using an JEOL JNM ECZL400S spectrometer operating at a field strength of approximately 9.4 Tesla (400 MHz for 1H nuclei). Approximately 5–10 mg of the purified sample was dissolved in deuterated solvent (DMSO-d_6) and transferred into a 5 mm NMR tube for analysis. The spectra were recorded at room temperature, and tetramethylsilane (TMS) was used as the internal reference standard. Chemical shifts were expressed in parts per million (ppm) relative to TMS. The obtained 1H was analysed to identify characteristic signals corresponding to aromatic protons, methoxy groups, lignan backbone protons, and glucose moieties present in the SDG structure. The observed chemical shifts and signal patterns were compared with reported literature data for SDG, and the agreement of spectral features was used to confirm the structural identity of the isolated compound.

Experimental Work

Instrumentation: Chromatographic analysis was performed using a Shimadzu Prominence UFLC RF-20A system equipped with a photodiode array (PDA) detector, solvent delivery module, auto-sampler, and column oven. The system allowed efficient separation and detection of analytes under optimized chromatographic conditions. Data acquisition, system control, and peak integration were performed using LabSolutions software. The PDA detector facilitated simultaneous monitoring of multiple wavelengths and provided spectral information for reliable identification and quantification of the analytes.

Preparation of Solutions

Mobile Phase A (0.1% Glacial Acetic Acid in Ethanol): Mobile phase A was prepared by adding 0.1% (v/v) glacial acetic acid to ethanol. Briefly, 1 mL of glacial acetic acid was transferred to a 1000 mL volumetric flask and the volume was made up with ethanol. The solution was sonicated for 10 minutes for degassing and filtered through a 0.45 µm membrane filter using vacuum filtration before use.

Mobile Phase B (0.1% Glacial Acetic Acid in Milli Q Water): Mobile phase B was prepared by adding 0.1% (v/v) glacial acetic acid to Milli-Q water. 1 mL of

glacial acetic acid was added to a 1000 mL volumetric flask and the volume was made up with Milli-Q water. The solution was sonicated for 10 minutes and filtered through a 0.45 µm membrane filter using vacuum filtration prior.

Diluent: Ethanol and Milli Q water in the ratio 0f 20: 80.

Stock Solution 1 (1000 µg/mL): Accurately 10 mg of secoisolariciresinol diglucoside (SDG) was weighed and transferred into a 10 mL volumetric flask. A small quantity of diluent was added and the solution was sonicated for about 10 minutes to ensure complete dissolution. The volume was then made up to the mark with diluent and mixed well to obtain Stock Solution I (1000 µg/mL).

Stock Solution 2 (100 µg/mL): From Stock Solution I, 1 mL was accurately pipetted and transferred into a 10 mL volumetric flask. The volume was made up to the mark with diluent and mixed thoroughly to obtain Stock Solution 2 (100 µg/mL), which was used as the working standard solution for chromatographic analysis.

Sample Preparation: A suitable quantity of flaxseed extract (10 mg) was accurately weighed and transferred into a 10 mL volumetric flask. The extract was dissolved in a small volume of diluent and sonicated for 10 minutes to facilitate complete extraction of the analytes. The volume was then made up to the mark with diluent and mixed well. The resulting solution was filtered through Millipore PVDF 0.45µ membrane filter.

Optimized Chromatographic condition:

Instrument	:	Ultra-Fast liquid chromatography (RF-20A)
Column	:	BDS Hypersil C18, (200 x 4.6) mm, 5 µm
Column temperature	:	30°C
Auto sampler temperature	:	25°C
Flow rate	:	1.0 mL/min
Wavelength	:	220 nm
Injection volume	:	10 µL
Run time	:	10 min
Mode of analysis	:	Isocratic
Blank	:	Diluent

Method Validation

Method validation in HPLC is a systematic process used to confirm that an analytical method is suitable for its intended purpose. It ensures that the method provides accurate, precise, and reliable results in compliance with regulatory guidelines (such as ICH Q2(R1)).

The developed HPLC method was validated following ICH guidelines to ensure reliability and reproducibility. Validation parameters included accuracy, assessed through recovery studies; precision, evaluated by intra- and inter-day variations; linearity, determined across a specified concentration range; and specificity, ensuring no interference from excipients or impurities. Sensitivity was confirmed through limit of detection (LOD) and limit of quantification (LOQ) measurements, while varying method parameters tested robustness to assess stability (Figure 4).

1. System Suitability: System suitability testing (SST) is a critical component of analytical method validation, ensuring that the analytical system is functioning correctly before sample analysis. It is performed before and during an analytical run to confirm the system's performance based on predefined parameters.

2. Linearity: Linearity evaluates the method's ability to produce results that are directly proportional to the analyte concentration over a specified range. It is determined by plotting the calibration curve and assessing the correlation coefficient (R^2).

3. Specificity: Specificity ensures that the method can accurately measure the analyte without interference from other components such as impurities, degradation products, or matrix effects.

4. Accuracy: Accuracy measures how close the test results are to the true value or standard. It is typically assessed using recovery studies by comparing known spiked concentrations with measured values.

5. Precision: Precision refers to the reproducibility of results under the same conditions. It is divided into:

Repeatability (intra-day precision) – Consistency within the same day.

Intermediate Precision – Variability across different days, analysts, or instruments.

Reproducibility – Precision across different laboratories.

6. Detection Limit (LOD): LOD is the smallest concentration of the analyte that can be detected but not necessarily quantified. It is typically calculated using the signal-to-noise ratio or standard deviation of the response.

7. Limit of Quantification (LOQ): LOQ is the lowest concentration that can be reliably quantified with acceptable accuracy and precision. It is often determined as a multiple of the LOD (e.g., 10 times the standard deviation of the response).

8. Robustness: Robustness assesses the method's ability to remain unaffected by small variations in experimental conditions, such as temperature, pH, or flow rate in chromatography.

Figure 1 shows procedure followed for extraction of SDG from Flax seed.

Phytochemical Analysis:

Preliminary phytochemical screening of the flaxseed extracts revealed the presence of several biologically active secondary metabolites. Qualitative phytochemical tests indicated that the extract contained phenolic compounds, flavonoids, terpenoids, steroids, anthocyanins, emodins and glycosides. These findings indicate that flaxseed is a rich source of phytochemicals, particularly phenolics, flavonoids and lignans such as secoisolariciresinol diglucoside (SDG), which contribute to its antioxidant and therapeutic potential.

Table 1. Phytochemical screening tests for flax seed extraction by ethanol

CONSTITUENT	Flaxseed extract
Alkaloids	-
Flavonoids	+
Phenolics	+++
Saponins	-
Terpenoids	+++
Glycosides	-
Emodin	++

Steroids	++
Anthocyanins	+
Tannins	-

(+++), (++), (+) and (-) refer to high, moderate, low and absent amounts respectively

Determination of total Phenolic content:

Total phenolic content of the flaxseed extract was determined using the Folin–Ciocalteu method with gallic acid as the reference standard. A calibration curve was prepared using gallic acid concentrations of 10–50 µg/mL, which showed absorbance values ranging from 0.096 to 0.486. The calibration plot demonstrated good linearity with the regression equation: $y = 0.0097x - 0.0004$, where y represents absorbance and x represents concentration (µg/mL). The absorbance of the flaxseed extract was recorded as 0.696, which corresponded to a phenolic concentration of approximately 71.4 µg/mL gallic acid equivalents (GAE). The result indicates that the flaxseed extract contains a substantial number of phenolic compounds, which play a major role in plant antioxidant activity and contribute significantly to health-promoting properties such as free-radical scavenging and anti-inflammatory effects.

Determination of Total Flavonoid Content:

The total flavonoid content of the flaxseed extract was determined using the standard colorimetric method with quercetin as the reference standard. A calibration curve was constructed using standard concentrations ranging from 10–50 µg/mL, which produced absorbance values between 0.073 and 0.372. The calibration curve showed good linearity with the regression equation: $y = 0.00746x - 0.0012$, where y represents absorbance and x represents concentration (µg/mL). The flaxseed extract exhibited an absorbance of 0.088, corresponding to a flavonoid concentration of approximately 11.6 µg/mL quercetin equivalents (QE). This result confirms the presence of flavonoid compounds in the extract, which may contribute to the biological activity of flaxseed.

Determination of SDG by ATIR spectroscopy:

The FT-IR spectra of the isolated compound were compared with the reference standard of secoisolariciresinol diglucoside (SDG) to identify the characteristic

functional groups of the lignan structure. The spectra exhibited prominent absorption bands corresponding to phenolic hydroxyl groups (~3300 cm^{-1}), aromatic C=C skeletal vibrations (~1600–1500 cm^{-1}), and glycosidic C–O stretching bands (~1100–1000 cm^{-1}). These functional groups are typical structural features of SDG. The observed peaks in the isolated sample closely matched those of the SDG standard, confirming the presence of secoisolariciresinol diglucoside (SDG) in the flaxseed extract.

Table 2. ATIR spectroscopy of SDG

Functional Group	**SDG Standard (cm^{-1})**	**Extracted Sample (cm^{-1})**	**Assignment**
O–H stretching	3326	3285	Phenolic and carbohydrate hydroxyl groups
C–H stretching	—	2921	Aliphatic C–H vibrations of lignan backbone
C=O stretching	—	1743	Carbonyl group (minor phenolic/associated compounds)
Aromatic C=C stretching	1601	1649	Aromatic benzene ring vibrations
Aromatic skeletal vibration	1514	1559	Characteristic lignan aromatic ring
O–H bending	1363	1378	Phenolic hydroxyl group vibration
C–O stretching	1268	—	Phenolic ether linkage
C–O–C stretching	1153	1075	Glycosidic linkage of glucose
C–O stretching	1072	996	Carbohydrate moiety
Aromatic C–H bending	796	878 / 946	Substituted benzene ring

Figure 2 shows calibration curve, IR Report of Standard SDG and IR report of extract.

Determination of SDG by NMR Spectroscopy:

The structure of the compound isolated from Linum usitatissimum was confirmed by ^{1}H NMR spectroscopy and comparison with the reference standard of secoisolariciresinol diglucoside (SDG). The ^{1}H NMR spectrum of the SDG standard showed characteristic signals corresponding to the aromatic protons of guaiacyl rings, anomeric protons of β-D-glucose units, sugar ring protons, and benzylic protons of the dibenzylbutane lignan backbone.

In the standard spectrum, the aromatic proton signals appeared in the region δ 6.81–6.54 ppm, corresponding to the H-2, H-5 and H-6 protons of the two guaiacyl aromatic rings present in the lignan structure. Two signals observed at δ 4.415 ppm and δ 4.395 ppm were assigned to the anomeric protons of the two β-D-glucopyranoside units, confirming the diglucoside nature of SDG. The sugar proton signals were observed as multiplets in the region δ 4.13–3.63 ppm, corresponding to protons of the glucose moieties. Signals appearing in the region δ 3.4–2.8 ppm correspond to the benzylic methine and methylene protons of the secoisolariciresinol backbone, while the signals in the region δ 2.5–1.9 ppm correspond to the central aliphatic protons of the lignan skeleton.

The ^{1}H NMR spectrum of the compound isolated from flaxseed extract exhibited similar characteristic signals, particularly in the aromatic region (δ 6.3–6.8 ppm) and the anomeric region (δ 4.6–5.2 ppm). The sugar proton signals were observed as a broad cluster in the region δ 3.2–3.9 ppm, indicating the presence of glucose moieties. Slight peak broadening and minor chemical shift variations were observed in the extract spectrum due to matrix effects and overlapping signals from co-extracted phytochemicals.

Overall, the presence of aromatic lignan signals, two anomeric protons indicating diglucoside linkage, and benzylic protons corresponding to the dibenzylbutane core confirmed that the isolated compound corresponds to secoisolariciresinol diglucoside (SDG).

Table 3. NMR Spectroscopy of SDG

Proton Assignment	Structural Position	Standard SDG (δ ppm)	Extracted Sample (δ ppm)	Interpretation
Aromatic protons	H-2, H-5, H-6 (guaiacyl rings)	6.81 – 6.54	6.30 – 6.80	Signals correspond to aromatic protons of the 1,3,4-substituted guaiacyl rings characteristic of lignan structure
Anomeric proton (Glucose-1)	H-1′	4.415	4.60 – 5.20	Indicates glycosidic linkage of β-D-glucose
Anomeric proton (Glucose-2)	H-1″	4.395	4.60 – 5.20	Confirms presence of two glucose units forming diglucoside
Sugar ring protons	H-2′ – H-6′, H-2″ – H-6″	4.13 – 3.63	3.20 – 3.90	Multiplet signals corresponding to glucose ring protons
Benzylic protons	H-7, H-7′	3.40 – 2.80	2.80 – 3.50	Protons adjacent to aromatic rings in lignan backbone
Central aliphatic protons	H-8, H-8′	2.50 – 1.90	2.00 – 2.70	Methine/methylene protons of the dibenzylbutane core

Figure 3 shows NMR Report of Standard SDG, NMR Report of extract and Chromatogram of standard SDG and Chromatogram of extract.

Method Development and validation:

Optimized Method:

The optimized Ultra-Fast Liquid Chromatography (UFLC) method was developed using a BDS Hypersil C18 column (200 × 4.6 mm, 5 µm) under isocratic

conditions with a flow rate of 1.0 mL/min, column temperature of 30°C, autosampler temperature of 25 °C, detection wavelength of 220 nm, injection volume of 10 µL, and a run time of 10 minutes using diluent as blank. Under these conditions, the analyte in the extract produced a sharp and well-resolved peak at a retention time of approximately 4.1 minutes, indicating efficient separation, while the use of environmentally benign solvents such as ethanol and water highlights the green and sustainable nature of the developed analytical method.

System Suitability:

System suitability of the method was evaluated by injecting six replicate injections of the standard solution of SDG at a concentration of 100 µg/mL. The chromatographic parameters such as peak area, retention time, tailing factor, and theoretical plates were assessed. The results showed consistent peak responses with %RSD values within the acceptable limit of ≤ 2%, indicating that the system performance was adequate for the analysis and the method is suitable for routine quantitative determination of SDG.

Table 4. System Suitability

SI NO.	RETENTION TIME	PEAK AREA	TAILING FACTOR	NTP
1.	4.090	808810	1.95	4.090
2.	4.080	804231	1.9	4.080
3.	4.083	807851	1.94	4.083
4.	4.075	805681	1.93	4.075
5.	4.068	806341	1.94	4.068
6.	4.083	806512	1.92	4.083
avg	4.080	806737.67	1.93	4079.83
SD	0.00716	0.01817	0.01817	7.14
%RSD	0.18	0.94	0.94	0.18

Linearity:

It is the ability to obtain results from experiments that are consistent with the analyte concentration in the sample. By using the linearity formula y=mx+c, the calibration curve's linearity was ascertained, and the slope was calculated. The calibration curve was produced in triplicate using the following six concentrations: 10, 20, 40,60, 80 and 100 µg/ml. The SDG calibration curve is shown in the below image.

Table 5. Linearity

SI NO.	Concentration (µg/mL)	Peak area of SDG
1	10	65395
2	20	170223
3	40	311677
4	60	474788
5	80	639335
6	100	809811
8	Regression equation	y = 8130.7x − 8219.6
9	Correlation coefficient (r^2)	0.9999

Precision:

Interday Precision:

The intraday precision of the developed UFLC method was evaluated by analyzing three replicate injections of SDG at three concentration levels (10, 60, and 100 µg/mL) within the same day. The %RSD values obtained for all concentration levels were found to be less than 2%, indicating good repeatability and precision of the developed method under the same operating conditions.

Table 6. Interday Precision

Injection No	Secoisolariciresinol diglucoside (Peak area)		
	10µg	**60µg**	**100µg**
1	65395	474788	809811
2	64825	469780	813072
3	65176	473124	807912
Average	65132	472564	810288
Standard Deviation	287.54	2550.53	2578.35

Intraday Precision:

The intraday precision was assessed by analyzing SDG at three concentration levels (10, 60, and 100 µg/mL) on three different days. The %RSD values obtained were within the acceptable limit of ≤ 2%, demonstrating that the developed UFLC method is precise and reproducible across different days.

Table 7. Intraday Precision

Injection No	Secoisolariciresinol diglucoside (Peak area)		
	10µg	**60µg**	**100µg**
Day 1	65132	472564	810288
Day 2	64890	470980	808285
Day 3	65210	474120	812145
Average	65077.33	472554.67	810239.33
Standard Deviation	168.42	1570.29	1930.62
% RSD	0.26	0.33	0.24

LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) for secoisolariciresinol diglucoside (SDG) were determined based on the standard deviation of the response (σ) and the slope of the calibration curve (S) in accordance with ICH guidelines. The values were calculated using the equations LOD = 3.3σ/S and LOQ = 10σ/S, where S is the slope obtained from the linear regression equation of the calibration curve (y = 8130.7x − 8219.6). The calculated LOD and LOQ values indicate that the developed UFLC method is sufficiently sensitive for the detection and quantification of SDG.

Table 8. LOD and LOQ

Parameter	**Value**
LOD (µg/mL)	0.33
LOQ (µg/mL)	1.00

Robustness:

The robustness of the method was evaluated by introducing small deliberate variations in chromatographic parameters, namely wavelength (218 nm and 222 nm) and flow rate (0.9 and 1.1 mL/min). The study was performed using a standard solution of SDG at a concentration of 100 µg/mL, and each condition was analyzed in triplicate injections. The chromatographic responses obtained under the varied conditions showed no significant changes in peak area and retention time, and the %RSD values were within the acceptable limit of ≤ 2%, indicating that the developed method is robust and unaffected by minor variations in analytical conditions.

Table 9. Robustness

SI NO.	Wave Length	Peak Area	Flow Rate Ml/Min	Peak Area
1.	218	868832	0.9	902484
2.		873104		895488
3.		868575		900758
avg		870170.33		899576.67
SD		2548.98		3620.62
%RSD		0.29		0.40
1.	222	750680	1.1	748651
2.		747585		748605
3.		746737		744514
avg		748334		747256.67
SD		2076.73		2391.16
%RSD		0.28		0.32

Accuracy and Recovery: The accuracy of the method was evaluated by performing recovery studies using the standard addition method. A sample solution containing 40 µg/mL of SDG was spiked with additional standard SDG at 50%, 100%, and 150% levels, and each level was analyzed in triplicate injections. The percentage recovery was calculated by comparing the measured concentration with the added amount of standard. The results showed satisfactory recovery values within the acceptable range of 98–102%, indicating that the developed method is accurate and free from interference from the sample matrix, and is suitable for the quantitative estimation of SDG.

Table 10. Accuracy and Recovery

Set	%Levels (About)	Area Response	mg added	mg recovered	% recovery	Mean % Recovery	% RSD
1	50%	567685	0.06	0.06	99.68	100.0	0.92
		565562	0.06	0.06	99.28		
		573642	0.06	0.06	101.04		
2	100%	710848	0.08	0.08	100.46	100.01	0.41
		705324	0.08	0.08	99.68		
		706872	0.08	0.08	99.90		
3	150%	832338	0.1	0.1	100.67	100.01	0.57
		823860	0.1	0.1	99.65		
		824410	0.1	0.1	99.72		

Figure 4 shows Calibration curve of SDG and Overlay graph of linearity concentrations

Forced Degradation Studies

Forced degradation studies were performed under acidic, alkaline, oxidative, thermal and photolytic stress conditions at a concentration of 80 µg/mL. The percentage assay was calculated by comparing the peak area of stressed samples with that of the unstressed sample. Minor degradation was observed under acidic conditions (93.63%), whereas other stress conditions showed assay values close to the unstressed sample, indicating that the developed HPLC method is capable of detecting degradation products and can be considered stability indicating

%Assay=Area of unstressed sample/Area of stressed sample×100

1. Acidic Degradation

Acid hydrolysis was carried out by adding 1 mL of 0.1 N hydrochloric acid (HCl) to the standard solution containing 80 µg/mL of analyte. The solution was kept at 40°C for 2 hours to induce degradation. After completion of the stress period, the solution was neutralized with 0.1 N sodium hydroxide, diluted appropriately with mobile phase, filtered through a 0.45 µm membrane filter, and injected for analysis.

2. Alkaline Degradation

For alkaline degradation, 1 mL of 0.1 N sodium hydroxide (NaOH) was added to the 80 µg/mL analyte solution. The mixture was maintained at 40°C for 2 hours. After the degradation period, the solution was neutralized using 0.1 N hydrochloric acid, diluted with mobile phase, filtered through a 0.45 µm membrane filter, and analyzed.

3. Oxidative Degradation

Oxidative degradation was carried out by treating the 80 µg/mL analyte solution with 1 mL of 3% hydrogen peroxide (H_2O_2). The mixture was maintained at 40°C for 2 hours to allow oxidative degradation to occur. After completion of the stress treatment, the solution was diluted with mobile phase, filtered through a 0.45 µm membrane filter and injected.

4. Thermal Degradation

Thermal degradation studies were performed by exposing the 80 µg/mL analyte solution to elevated temperature. The solution was placed in a hot air oven maintained at 80°C for 2 hours. After the stress exposure, the solution was cooled to room temperature, diluted with mobile phase, if necessary, filtered through a 0.45 µm membrane filter, and analyzed.

5. Photolytic Degradation (UV Study)

For photolytic degradation, the 80 µg/mL analyte solution was exposed to UV light in a photostability chamber. The solution was kept at 40°C for 2 hours under UV irradiation. After exposure, the sample was diluted with mobile phase if required, filtered through a 0.45 µm membrane filter, and injected for analysis

Table 11. Forced Degradation Studies

Stress Condition	SDG			
	% Assay	% Impurity	Mass Balance (NLT 95.0)	Purity Flag
Unstressed sample	100	0.00	Pass	No impurity
Photolytic	104.95	0.00	Pass	pure
Acid	93.63	6.37	Fail	Minor Degradation
Base	106.93	0.00	Pass	Pure
Peroxide	97.85	2.15	Pass	Pure
Thermal	107.61	0.00	pass	Pure

Figure 5 shows Chromatogram of Unstressed sample, Chromatogram of Photolytic degradation, Chromatogram of Acid degradation, Chromatogram of Base degradation, Chromatogram of Peroxide degradation and Chromatogram of Thermal degradation.

Conclusion:

This study successfully demonstrated a green and efficient approach for the extraction, isolation, characterization, and quantitative analysis of secoisolariciresinol diglucoside (SDG) from flaxseed (Linum usitatissimum L.). The use of environmentally friendly solvents such as ethanol and water, combined with microwave-assisted extraction, provided an effective and sustainable method for obtaining lignan-rich extracts. Preliminary phytochemical screening confirmed the presence of important secondary metabolites including phenolics, flavonoids, terpenoids, and lignans, indicating the rich phytochemical composition of

flaxseed. Quantitative analysis revealed a significant content of phenolic and flavonoid compounds, supporting the antioxidant potential of the extract.

The isolated compound was successfully characterized using FT-IR and ^{1}H NMR spectroscopy, and the obtained spectral data were found to be consistent with the reference standard of secoisolariciresinol diglucoside, confirming the structural identity of the isolated lignan. A green UFLC analytical method was developed and optimized for the determination of SDG using ethanol–water based mobile phases. The method showed excellent chromatographic performance with a retention time of approximately 4.1 minutes, indicating efficient separation. Validation of the method according to ICH guidelines demonstrated good linearity ($R^2 = 0.9999$), precision (%RSD < 2%), accuracy (98–102% recovery), sensitivity (LOD 0.33 µg/mL and LOQ 1.0 µg/mL), and robustness, confirming the reliability of the developed method.

Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions showed that the method is capable of detecting degradation products, thereby establishing it as a stability-indicating analytical method.

Overall, the study provides a simple, reliable, and environmentally sustainable analytical strategy for the extraction and quantification of SDG from flaxseed, which may support further research on lignan-based nutraceuticals and their potential role in metabolic disorders such as PCOS.

I claim,

1. A method for extraction of secoisolariciresinol diglucoside (SDG) from flaxseed, comprising:

a) defatting flaxseed into meal and grounding into a fine powder;

b) preparing extraction solvent using ethanol-water solvent (50–70% v/v) with 1–5% Tween 80, mixing in ratio 1:20 (w/v);

c) mixing powdered defatted flaxseed meal of step a) with the prepared extraction solvent of step b) in a solid-to-solvent ratio of approximately 1:20 (w/v);

d) facilitating microwave-assisted extraction at 25–30°C for 30 minutes;

e) processing with water bath treatment, filtration and drying.

wherein microwave-assisted extraction enhances release of lignan compounds.

wherein lignan-rich extract contains secoisolariciresinol diglucoside.

2. A method for isolation of secoisolariciresinol diglucoside (SDG) from flaxseed, comprising:

a) powdering clean and dry seeds of flaxseed;

b) defatting the powder using hexane;

c) extracting using 1,4-dioxane:ethanol (1:1 v/v) under continuous stirring;

d) hydrolyzing the extract with methanolic NaOH (20 mM, pH 8) at 50°C;

e) acidifying the extract to pH 3 using dilute sulfuric acid; and

f) purifying the extract using silica gel column chromatography.

3. A Ultra-Fast Liquid Chromatography (UFLC) method for determination of SDG, comprising:

a) dissolving ethanol and water with 0.1% glacial acetic acid as mobile phase;

b) running chromatogram through column using BDS Hypersil C18 column, flow rate 1.0 mL/min, wavelength 220 nm using mobile phase of step a);

c) optimizing conditions at injection volume 10 µL, run time 10 minutes and retention time approximately 4.1 minutes.

d) running the sample of step c) and recording chromatogram from the chromatograph for estimation of SDG.

4. The Ultra-Fast Liquid Chromatography (UFLC) method for determination of SDG as claimed in claim 4, wherein SDG is obtained at Rf value approximately 0.52;

5. The Ultra-Fast Liquid Chromatography (UFLC) method for determination of secoisolariciresinol diglucoside (SDG) as claimed in claim 3, wherein the characterization of SDG using Fourier Transform Infrared (FT-IR) spectroscopy shows absorption bands in the range of 3285–3326 cm^{-1}, 1514–1649 cm^{-1}, and 996–1153 cm^{-1}; and wherein characterization using ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy shows signals in the range of δ 6.30–6.81 ppm, δ 4.39–5.20 ppm, δ 3.20–4.13 ppm, δ 2.80–3.50 ppm, and δ 1.90–2.70 ppm.

6. The Ultra-Fast Liquid Chromatography (UFLC) method for determination of SDG as claimed in claim 3, wherein the method showing linearity in the range 10–100 µg/mL with regression equation $y = 8130.7x - 8219.6$ and correlation coefficient 0.9999; precision with %RSD ≤ 2%; accuracy with recovery 98–102%; LOD 0.33 µg/mL and LOQ 1.00 µg/mL and robustness with %RSD ≤ 2%.

7. The Ultra-Fast Liquid Chromatography (UFLC) method for determination of SDG as claimed in claim 3, wherein forced degradation under acidic, alkaline, oxidative, thermal and photolytic conditions indicates stability-indicating capability.

8. The Ultra-Fast Liquid Chromatography (UFLC) method for determination of SDG as claimed in claim 3, wherein the study provides a simple, reliable, and environmentally sustainable analytical strategy for the extraction and quantification of SDG from flaxseed, which may support further research on lignan-based nutraceuticals and their potential role in metabolic disorders such as PCOS.

Dated this 9th day of May, 2026

To be signed digitally by
(Sanchita Tewari)
Agent for the Applicant
Patent Agent (IN/PA 2711)

ABSTRACT

GREEN EXTRACTION OF SDG FROM FLAXSEED: DEVELOPMENT OF A SUSTAINABLE ANALYTICAL METHOD

The present invention discloses a green and efficient method for extraction, isolation, characterization and quantitative analysis of secoisolariciresinol diglucoside (SDG) from flaxseed (*Linum usitatissimum* L.). The extraction is carried out using ethanol-water (50–70% v/v) and Tween 80 (1–5% v/v) followed by microwave-assisted extraction at 25–30°C for 30 minutes, water bath treatment, filtration and drying to obtain lignan-rich extract. Isolation is performed using 1,4-dioxane:ethanol (1:1 v/v), hydrolysis with methanolic NaOH (20mM, pH 8), acidification and column chromatography to obtain SDG with Rf value approximately 0.52. The characterization of SDG using Fourier Transform Infrared spectroscopy shows absorption bands ranging 3285–3326 cm^{-1}, 1514–1649 cm^{-1}, and 996–1153 cm^{-1}, corresponding to hydroxyl, aromatic, and glycosidic functional groups, respectively. The ^{1}H Nuclear Magnetic Resonance spectroscopy shows signals in the range of δ 6.30–6.81 ppm, δ 4.39–5.20 ppm, δ 3.20–4.13 ppm, δ 2.80–3.50 ppm, and δ 1.90–2.70 ppm, confirming the presence of aromatic protons, anomeric protons, sugar moieties, and lignan backbone of SDG. The present invention provides an UFLC method for determination of SDG using BDS Hypersil C18 column with retention time approximately 4.1 minutes shows linearity (10–100 µg/mL, $r^2 = 0.9999$), precision (%RSD ≤ 2%), accuracy (98–102%), LOD 0.33 µg/mL and LOQ 1.00 µg/mL and stability-indicating capability under forced degradation conditions.