Scientific paper

Validation of an Isocratic HPLC Method for Simultaneous Estimation of Major Phytosterols in *Prunus spinosa* L. Extracts

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Abstract

This study aimed to develop a rapid method for the separation of stigmasterol, campesterol and β-sitosterol in *Prunus spinosa* L. (blackthorn) fruit extracts by HPLC system. Samples were prepared by Soxhlet extraction method and separated on a C18 column using acetonitrile-methanol mobile phase and photodiode array detector (PDA). The optimized method resulted in a linear calibration curve ranging from 1.70–130 μg mL^{-1} for all three phytosterols. Analyses of external phytosterol standards showed good linearity (R^2 of 0.998 to 0.999); LOD and LOQ were determined to be 0.32–9.30 μg mL^{-1} and 0.98–28.1 μg mL^{-1}, respectively. Repeatability and reproducibility precision analyses showed acceptable values of %RSD. β-sitosterol was the predominant phytosterol (51.53–81.03% of total) among all samples. Method validation parameters indicated that this analytical method can be applied for accurate and precise determination of campesterol, stigmasterol and β-sitosterol, in selected extracts.

Keywords: phytosterols; *Prunus spinosa* L. ethanolic extracts; HPLC; method validation.

1. Introduction

Prunus spinosa L., called blackthorn or sloe (Fig. 1), is a specie of flowering plant from the Rosaceae family. Blackthorn is a rather bushy tree with dark branches, hence the "black" thorn. In early spring it produces a five-petalled white flower, which is then replaced by deep blue-purple fruits. It blooms from March to April and ripens in October. Blackthorn is native to Europe and western Asia, but can also be found in New Zealand and eastern North America. Traditionally, blackthorn flowers and fruits have been used in various medicines, including tonics and syrups that "purify the blood", aid digestion and relieve rheumatism. Flowers, bark, leaves and fruits are astringent, depurative, diaphoretic, diuretic, laxative and stomachic.[1] Infusion of flowers is used in the treatment of diarrhoea (especially in children), bladder and kidney diseases.[2] Although not specifically mentioned for this species, all members of the genus contain amygdalin and prunasin, substances that break down in water to form hydrocyanic acid (cyanide or prussic acid). In small amounts, this highly toxic compound stimulates breathing, improves digestion and gives a sense of well-being.[3]

Phytosterols are a group of cholesterol-like compounds found naturally in plants. They differ from cholesterol by their carbon side chains and the presence or absence of double bonds. So far, between 200–300 different types of phytosterols have been successfully isolated and identified in botanical sources, where campesterol, stigmasterol and β-sitosterol (Fig. 2) have been found to be dominant and most frequently identified.[4] Phytosterols have attracted much attention due to their nutritional properties and biological effects such as inhibition of intestinal cholesterol absorption, lowering of blood low-density lipoprotein (LDL), anti-inflammatory and anti-cancer effects. Today, they are widely used in pharmaceutical products, nutritional supplements and cosmetics.[3–6]

Fig. 1. *Prunus spinosa* L. leaves, flowers and fruit

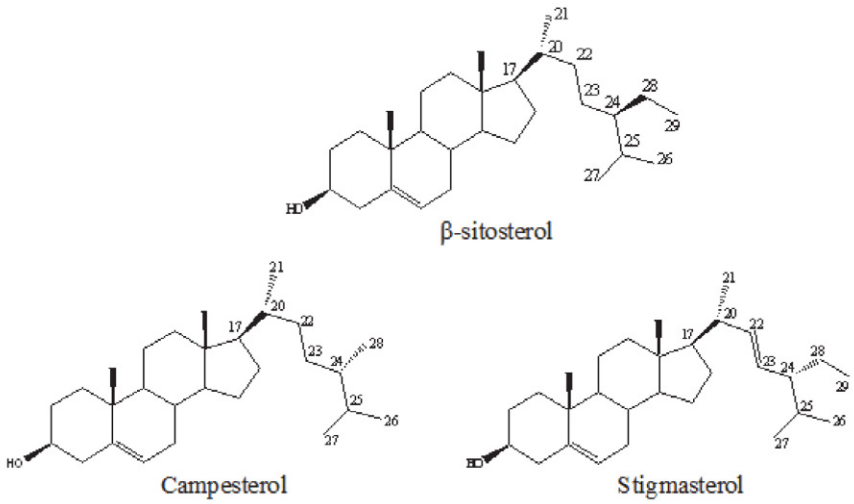

Fig. 2. Structural formulae of *Prunus spinosa* L. major phytosterols

The official methods for the separation and quantitative analysis of phytosterols are conventionally based on gas chromatography (GC). However, it usually requires chemical derivatization for a favourable peak shape, better sensitivity and resolution, and higher stability for labile unsaturated sterols.[7] In general, liquid chromatography (LC) has better operating conditions, including lower temperatures and pressure conditions for column separation, as well as several available detectors and has been widely used for sterol separation.[8–12] Rocco and Fanali[13] developed a nano-LC method for the determination of stigmasterol, campesterol and *β*-sitos-

terol in extra virgin olive oil, with good sensitivity and precision. Zarrouk et al.[14] and Fibigr et al.[15] reported on LC methods successfully applied to direct determination of sterols and lipids.

Until now, no studies on HPLC use for the separation of phytosterols from plant material and plant extracts in Bosnia and Herzegovina have been reported. The present study aimed to develop and validate a simple and rapid method for simultaneous separation of three major phytosterols, campesterol, stigmasterol and β-sitosterol, in plant extracts using HPLC system coupled to PDA detection. Furthermore, it was efficiently validated and used in phytosterol analysis in ethanolic extracts of *Prunus spinosa* L. fruit.

2. Materials and Methods

2. 1. Chemicals and Reagents

All reagents used in this study were of analytical grade. Standard compounds campesterol (99.6%), stigmasterol (98.1%) and β-sitosterol (98.6%) were purchased from Sigma-Aldrich, Chemie GmbH, Germany. Acetonitrile (ACN), ethanol (EtOH) and methanol (MeOH) were of HPLC grade and were obtained from Merck, Darmstadt, Germany. High-purity deionized water was prepared by a Milli-Q ultrapure purification system (Millipore, Billerica, Massachusetts, USA).

2. 2. Plant Material

Prunus spinosa L. plant material was collected from three different locations in Bosnia and Herzegovina (Borije, altitude 892 m, N43°51′16.60″, E18°28′55.33″, Vareš, altitude 739 m, N44°04′40.82″, E18°14′46.41″ and Trnovo, altitude 935 m, N43°41′19.37″ E18°22′34.39″) from March until November. Plant specimen was authenticated at the Department of Biology, Faculty of Science-University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

2. 3. Sample Preparation

Blackthorn fruits were washed well, using tap water and distilled water. Extracts were prepared using Soxhlet extraction with ethanol as a solvent. In a Soxhlet apparatus, 100 g of fresh fruits were extracted at the boiling point of the solvent for 6 h. It was performed 12 extractions in total. The volumes of obtained extracts were reduced in a rotary evaporator (RV-10, IKA, Sigma Aldrich, Deutschland) to approximately 5–7 mL and evaporated to crude extracts in a vacuum concentrator (Thermo Fisher Savant, SPD1010, SpeedVac Concentrator). Extracts in triplicate were stored in glass vials at $T \approx 4.0$ °C and used for the quantification of phytosterols and further determination of biological activities.

2. 4. Standard Solutions and Calibration

The working standard solutions of sterols were prepared by dissolving the respective mass of phytosterols standard compounds in ethanol. A linear regression equation was prepared from seven increasing concentrations by diluting the stock solution in ethanol. A linear relationship between peak area and concentrations (1.70–130 μg mL^{-1}) was obtained, and the linear regression equation for each standard was used for phytosterol quantification in standard solutions during the method validation process and phytosterol quantification in samples.

Tab. 1. Optimization of chromatographic conditions

Mobile phase	Ratio [%, *v/v*]	Mode	Column	Flow rate [mL min^{-1}]	λ [nm]	Temp. [°C]	t_R [min] S	t_R [min] C	t_R [min] B
ACN	100	Isocratic	Symmetry C18 (4.6 mm × 150 mm, 5 μm)	1.00	210	30	16.98	17.9	19.26
MeOH	100	Isocratic	Symmetry C18 (4.6 mm × 150 mm, 5 μm)	1.00	210	30	13.10	13.45	14.00
ACN:MeOH	90:10	Isocratic	Symmetry C18 (4.6 mm × 150 mm, 5 μm)	1.00	210	30	17.32	18.19	19.66
ACN:MeOH*	80:20	Isocratic	Symmetry C18 (4.6 mm × 150 mm, 5 μm)	1.00	210	30	15.46	16.18	17.45
ACN:EtOH	95:5	Isocratic	Symmetry C18 (4.6 mm × 150 mm, 5 μm)	1.00	210	30	16.98	17.6	19.3
ACN:EtOH	60:40	Isocratic	Inertsustain C18 (4.6 mm × 250 mm, 5 μm)	1.00	210	30	11.45	11.51	12.65
ACN:EtOH	40:60	Isocratic	Inertsustain C18 (4.6 mm × 250 mm, 5 μm)	1.00	210	30	11.48	11.51	12.66
ACN:water	95:5	Isocratic	Inertsustain C18 (4.6 mm × 250 mm, 5 μm)	1.00	278	40	15.46	16.11	17.60

* Optimized conditions for chromatographic separation; t_R= Retention time; S-stigmasterol; C-campesterol; B- β-sitosterol.

2. 5. HPLC System and Conditions

Chromatographic conditions were optimized using different columns, flow rates and mobile phase compositions given in Tab. 1. In order to find the most suitable mobile phase and the most appropriate chromatogram for detection and separation of phytosterols in blackthorn extracts, it was necessary to optimize the method regarding the composition of the mobile phase. Several different mobile phases were applied because those used in the study of Kakade and Magdum (2012)[16] were not reproducible for the samples tested in this research. After optimization, the final solvent system ACN:MeOH (80:20, *v/v*) and a flow rate of 1.00 mL min^{-1} in the isocratic mode were selected because these gave good resolution and shape of the chromatographic peaks for the components of interest.

Phytosterols were qualitatively and quantitatively analysed using an Agilent HPLC system series 1200 (Agilent Technologies, USA), equipped with a solvent delivery unit, autosampler and column oven. A PDA detector was used to collect chromatograms and UV spectra. Chromatographic separation was performed using a Symmetry C18 column (Waters, USA) (150 × 4.6 mm, 5 µm) at 30.0 °C and an injection volume of 20 µL. The mobile phase was filtered through a 0.45 µm hydrophilic polypropylene membrane filter and degassed in an ultrasonic bath prior to HPLC injection. Quantification of campesterol, stigmasterol and *β*-sitosterol was performed using a PDA detector set at 210 nm. Identification and quantification of chromatographic peaks were confirmed by comparison of the retention time (t_R) of extracts and phytosterol standards. Calibration curves were constructed by analysing seven concentrations of phytosterol standards ranging from 1.70 to 130 µg mL^{-1}. All analyses were performed at least in triplicate.

2. 6. Validation of the HPLC Analytical Method

Method validation was performed according to the ICH (2013) guidelines.[17] Compound identification was based on retention time matching and co-injection with authentic standards under identical analytical conditions.

Linearity: The linearity range was evaluated by plotting the relative peak area of phytosterol versus the relative concentration. For phytosterol analysis, standard solutions were prepared in ethanol in concentration ranges of 1.70–130 µg mL^{-1} for campesterol, stigmasterol and *β*-sitosterol. Three replicates were made for each of the seven solutions prepared. The correlation coefficient (R^2) was calculated for linearity evaluation. *LOD* and *LOQ* were calculated according to the following equations:

$$\frac{3.3 \times SD}{S} \quad (1)$$

$$LOQ = \frac{10 \times SD}{S} \quad (2)$$

where, S is the slope of the calibration curve and SD is the standard deviation of the response (n = 10). The following method validation criteria were applied: curve equation (y = mx + b), correlation coefficient ($R \geq 0.999$), coefficient of determination ($R^2 \geq 0.980$).[18–21]

Precision: Repeatability and reproducibility, two different levels of precision, were determined. Repeatability (intraday precision) was obtained by analysing standard solutions of phytosterols five times in the same day in triplicate. The same standard solutions were analysed five times in five independent days (once per day in triplicate) to obtain reproducibility (interday precision). Mean values, standard deviation and coefficient of variation were determined. The precision of the phytosterol analysis was assessed by the calculated relative standard deviation-RSD (recommended %RSD ≤ 3.9%).[18–21]

Accuracy: The accuracy of phytosterol analysis was evaluated by performing a recovery test. All phytosterols (campesterol, stigmasterol and β-sitosterol) were added to *P. spinosa* extract samples at three different concentration levels (25, 50, 75 µg mL^{-1}). Spiked samples were then analysed and recovery was calculated by the following equation:

$$Recovery\ (\%) = \frac{S_{total\ phytosterols} - S_{phytosterols\ present}}{S_{spiked\ phytosterol}} \times 100 \quad (3)$$

where, $S_{\text{total phytosterols}}$ = total amount of specific analysed phytosterol in extract sample,
$S_{\text{phytosterols present}}$ = amount of specific phytosterol present in extract sample,
$S_{\text{spiked phytosterol}}$ = spiked amount of specific phytosterol.

2. 7. Quantification of Phytosterols from Ethanolic Extracts of Blackthorn Fruit

The optimized HPLC method was used to estimate the phytosterols content in ethanolic extracts of *P. spinosa* L. fruit collected from three different locations in Bosnia and Herzegovina. The solutions of each crude extract were prepared separately (10 mg mL^{-1}) in ethanol, and sample of 20 µL was subjected to HPLC analysis. The peak area responses were recorded under the optimized and validated chromatographic condition. The phytosterols content expressed as mass concentration was determined from the linear regression equations. The identification of campesterol, stigmasterol and *β*-sitosterol in extracts was carried out by comparison of t_R for phytosterol standard solutions and samples. Samples of standard solutions and extracts were stored in a dark and cold place ($T \approx 4.0$ °C) to avoid oxidative degradation.

3. Results and Discussion

3. 1. Optimization of Chromatographic Conditions – HPLC Method Development and Validation

In this work, a method based on HPLC separation combined with PDA detection has been optimised, vali-

dated and applied for phytosterol analysis in *Prunus spinosa* L. ethanolic extracts. An isocratic elution was chosen since it is simple, requires only one pump and minimizes the variation of baseline and ghost peaks. Various columns are available for HPLC systems, but the Symmetry C18 column (150 × 4.6 mm, 5 μm) was preferred because peak shape, selectivity, and resolution provided the best results using this column. Among the different mobile phases used, acetonitrile:methanol (80:20, *v/v*) was suitable for the analysis of campesterol, stigmasterol and *β*-sitosterol (Tab. 1), due to baseline normalisation and the best separation of the each of three phytosterols (Fig. 3). Furthermore, a flow rate of 1 mL min^{-1} and an injection volume of 20 μL, with UV detection at 210 nm, provided optimal conditions for the analysis of these phytosterols (Tab. 2).

Although various stationary phases and several isocratic programs were tested, at least two phytosterols were not separated using the columns except for Symmetry C18, on which sterol standard solutions were fully separated. Different co-solvents (methanol, ethanol, acetonitrile, and water) and/or ratios were evaluated to enhance the separation of the three phytosterols. The results showed that the best peak shape and resolution were achieved when acetonitrile/methanol, 80:20, *v/v* mixture was applied, with an isocratic elution mode.

Table 2. HPLC system parameters for an optimised analytical method

Column	Symmetry C18, 150×4.6 mm, 5 μm particle size
Flow rate	1 mL min^{-1}
Mobile phase	Acetonitrile:methanol (80:20, *v/v*)
Run time	50 min
Wavelength	210 nm
Temperature	30 °C
Injection volume	20 μL
Sample solvent	Methanol

With optimized method conditions analytes were very good separated, with resolution values between the main peaks ranging from 0.97 to 1.43 (Tab. 3). The flow rate and column temperature were maintained at 1.00 mL min^{-1} and 30.0 °C, respectively.

The other mobile phases showed good results toward detected compounds too, but baseline separation was not reached and resolution was not appropriate.

The linearity of the HPLC method was investigated within the range of 1.70–130 μg mL^{-1} using seven different solutions with increasing concentrations. The calibration curves for investigated phytosterols were linear, with ex-

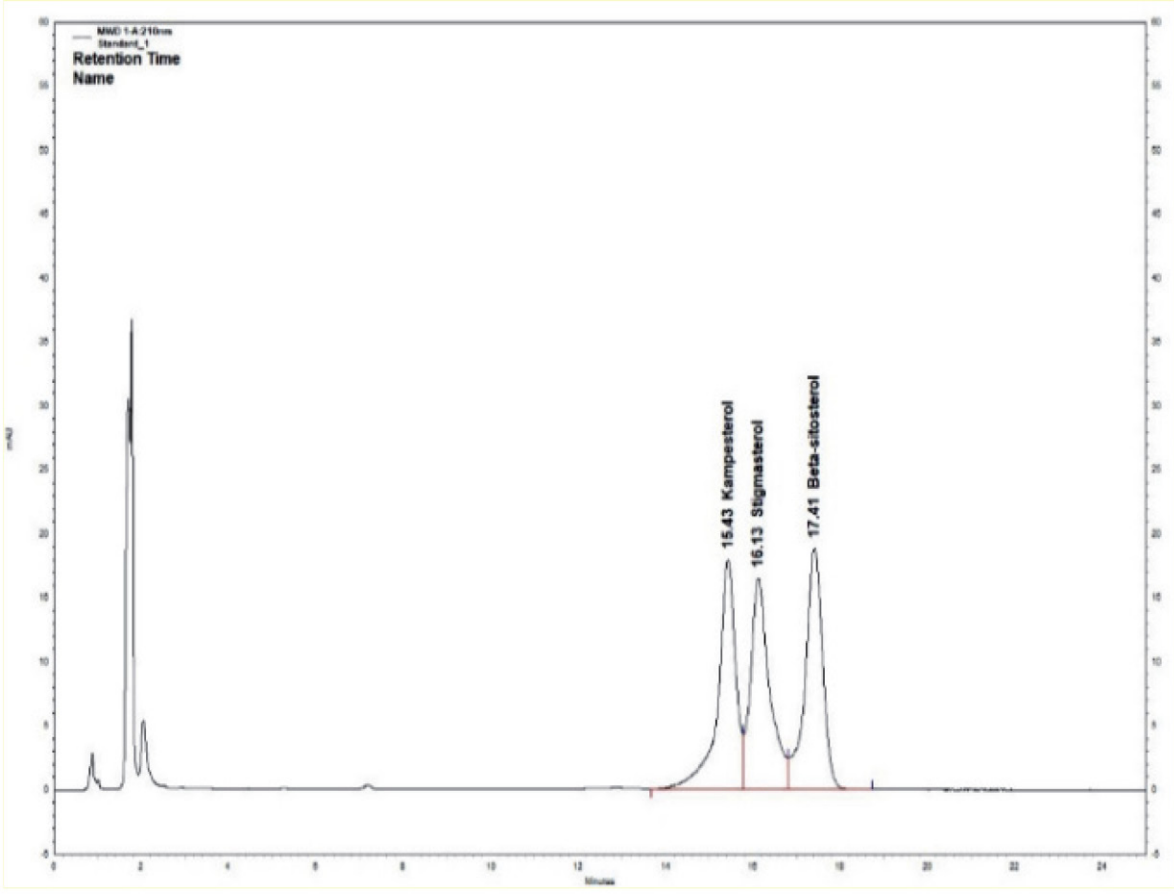

Fig. 3. HPLC chromatogram for phytosterol standards (*β*-sitosterol, campesterol and stigmasterol) on Symmetry C18 column with mobile phase: ACN:MeOH (80:20, *v/v*)

cellent correlation coefficients ranging from 0.998 to 0.999 (Tab. 3). The results further revealed that the minimum concentration levels at which the analyte can be reliably detected (*LOD*) and quantified (*LOQ*) were 0.32 μg mL^{-1} and 0.98 μg mL^{-1} for *β*-sitosterol, 9.30 μg mL^{-1} and 28.1 μg mL^{-1} for campesterol, 0.58 μg mL^{-1} and 1.75 μg mL^{-1} for stigmasterol, respectively, demonstrating good sensitivity of the method.

The system precision results indicated that the %RSD values were within the prescribed limit of %RSD < 2%, with the %RSD values of the peak area being 1.27% for *β*sitosterol, 0.98% for campesterol, and 0.56% for stigmasterol (Tab. 4). Similarly, the results obtained for the system precision showed that the %RSD values of the retention time were 1.15% for *β*sitosterol, 0.97% for campesterol, and 0.99% for stigmasterol (Tab. 4). The method precision results indicate that the %RSD values were also within the prescribed limit of %RSD < 2%, ranging from 1.03% for stigmasterol, 1.15% for campesterol, and 1.44% for *β* sitosterol, as shown in Tab. 4.

The precision of the validated method was expressed as repeatability and reproducibility with %RSD, and accuracy was evaluated by the recovery test. The %RSD values for intra-assay precision (repeatability) were in the range of 1.55% for stigmasterol to 2.50% for *β*-sitosterol (Tab. 5). Whereas the %RSD values for inter-assay (reproducibility) precision were in the range of 0.13% for *β*-sitosterol to 2.56% for campesterol (Tab. 5). These observations suggest that the method provides highly reproducible results, as shown in Tab. 5.

3. 2. Phytosterol Separation, Identification and Quantification in *P. Spinosa* L. Fruit Ethanolic Extracts

To assess the applicability of the validated method to the analysis of *P. spinosa* L. extracts, three ethanolic fruit extracts were obtained and analysed under the above established conditions. One of the goals of the work was to perform a chemical characterization of extracts using the HPLC method in terms of determining the presence of phytosterols, considering their important role in the human body. The presence of *β*-sitosterol was determined and quantified in all extracts of blackthorn fruits, while stigmasterol content was not detected in all samples. Compared to the other two quantified phytosterols, *β*-sitosterol stands out for its content.

Table 3. Calibration curve equations, t_R, R^2, *LOD* and *LOQ* values for phytosterols

Phytosterols	t_R [min]	Equations of Calibration Curves	R^2	*LOD* [μg mL^{-1}]	*LOQ* [μg mL^{-1}]	Resolution
Campesterol	15.43	$y = 8\times10^6x - 2.06\times10^4$	0.9984	9.30	28.1	0.97
Stigmasterol	16.13	$y = 8\times10^6x + 1.39\times10^3$	0.9995	0.58	1.75	1.43
β-sitosterol	17.41	$y = 1\times10^7x + 3.32\times10^3$	0.9996	0.32	0.98	

– In the calibration curve $y = mx + b$, y is the integrated peak area and x is the concentration; t_R – retention time, R – correlation coefficient; *LOD* – limit of detection; *LOQ* – limit of quantification.

Table 4. System and method precision data.

Parameter	System precision						Method precision		
	*β*sitosterol [10.7 μg mL^{-1}]		Campesterol [10.7 μg mL^{-1}]		Stigmasterol [10.7 μg mL^{-1}]		*β*sitosterol [10.7 μg mL^{-1}]	Campesterol [10.7 μg mL^{-1}]	Stigmasterol [10.7 μg mL^{-1}]
	Peak Area	t_R	Peak Area	t_R	Peak Area	t_R	Peak Area	Peak Area	Peak Area
Mean	70068.00	17.41	60251.00	15.43	113558.00	16.13	69180.00	61250.00	112929.00
SD	888.00	0.20	590.00	0.15	638.00	0.16	995.50	707.40	1163.20
%RSD [%]	1.27	1.15	0.98	0.97	0.56	0.99	1.44	1.15	1.03

$n = 6$; SD – standard deviation; RSD – relative standard deviation; t_R – retention time in min.

Tabable 5. Precision of method expressed as repeatability and reproducibility with %RSD

Phytosterols	t_R [min]	Linearity [mg mL^{-1}]	Repeatability %RSD	Reproducibility %RSD	Recovery [%]	%RSD
Campesterol	15.43	0.0017–0.13	2.18	2.56	99.86–100.5	0.05–0.40
Stigmasterol	16.13	0.0017–0.13	1.55	0.38	99.77–100.2	0.02–0.08
β-Sitosterol	17.41	0.0017–0.13	2.50	0.13	99.48–100.2	0.01–0.48

$n = 3$; *RSD* – relative standard deviation.

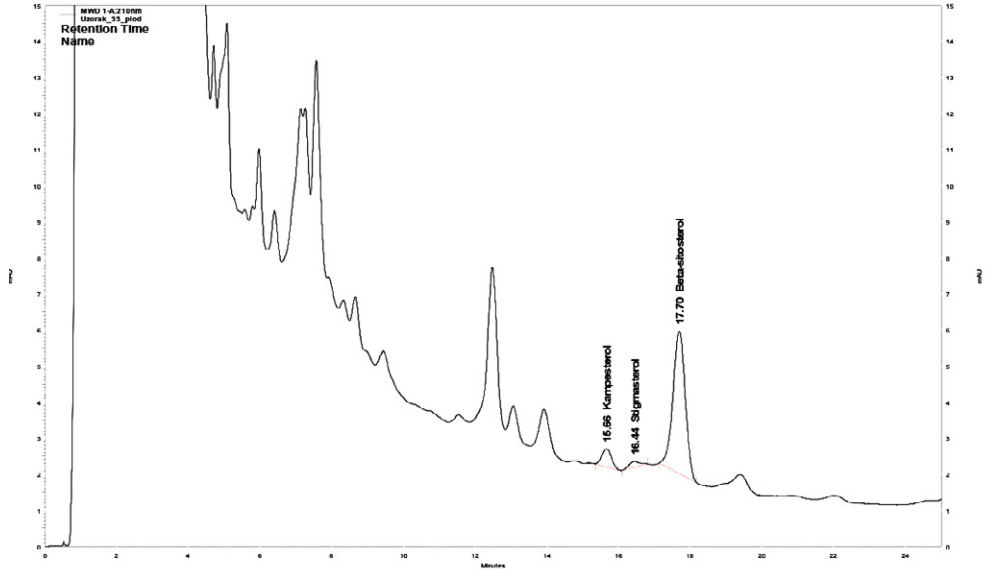

Fig. 4. Chromatogram of the ethanolic extract of fresh blackthorn fruits from the Borije location obtained by Soxhlet extraction

Fig. 4. presents a characteristic chromatogram of the extract mixture where phytosterols of interest were identified by comparison of retention times with standard solutions of phytosterols. HPLC chromatograms of campesterol, stigmasterol and β-sitosterol standard solutions were obtained under optimized chromatographic conditions by injecting 20 μL of solution. Retention times for campesterol, stigmasterol and β-sitosterol were found to be 15.43, 16.13 and 17.41 min, respectively, which were in good agreement with data reported for the standard solutions.

The content of the three main phytosterols in selected samples is summarized in Tab. 6. Fruit extracts from location Borije had the highest content of total sterols (campesterol, stigmasterol, and β-sitosterol) of 18.475 mg g^{-1}, then extracts from Trnovo 8.774 mg g^{-1}, while the extracts from Vareš had the lowest content of 3.82 mg g^{-1}. The content of β-sitosterol in the sample from Borije was 14.765 mg g^{-1}, and the content of campesterol was 3.250 mg g^{-1}. The content of β-sitosterol, campesterol and stigmasterol in the extracts of fresh blackthorn fruit from the location of Trnovo is significantly lower compared to the extracts from the location of Borije. As expected, β-sitosterol was the dominant phytosterol, and campesterol was present at a lower level. Stigmasterol was found in two of three samples at very low levels, which was in accordance with the results presented in previous studies concerning the content of phytosterols in different plant samples.[7,9]

Based on the results shown in Tab. 6, it can be observed that the ethanolic extracts obtained by SE from all three localities had a high content of phytosterols, among which the extract from the Borije location is the leading one. There is no data available in the literature on the specific content of phytosterols (β-sitosterol, campesterol and stigmasterol) in the ethanol extracts of *Prunus spinosa* L., so the obtained values cannot be compared with the results of other studies. The importance of determining phytosterols in plant extracts is related to their biological activities. One of the most important effects of phytoster-

Table 6. Estimation of campesterol, stigmasterol and β-sitosterol in blackthorn fruit ethanolic extracts from three locations in Bosnia and Herzegovina

Sample location	Campesterol		Stigmasterol		β-Sitosterol		Sum of determined phytosterols
	Peak area mean	Content [mg g^{-1}]±SD	Peak area mean	Content [mg g^{-1}]±SD	Peak area mean	Content [mg g^{-1}]±SD	Content [mg g^{-1}]±SD
Borije	18231	3.250±0.000*	6490	0.460±0.012	187753	14.765±0.007	18.475
Vareš	3873	1.850±0.002	–	–	26013	1.970±0.005	3.820
Trnovo	1892	1.641±0.010	1502	0.023±0.004	92164	7.110±0.013	8.774

Values are expressed as the mean of three determinations ± standard deviation.
*All standard deviation values less than 0.001 mg g^{-1}.

ols is antiproliferative activity. Previously published data suggest that the content of phytosterols in the diet is associated with a reduction in common cancers, including colon, breast and prostate cancers.[22,23] Phytosterols affect host systems and potentially enable a stronger antitumor response. This includes the recognition of cancer and strengthening the immune response, influencing the hormonally dependent growth of endocrine cancers and changing the way of sterol biosynthesis. In addition, phytosterols have direct inhibitory effects on cancer growth, including slowing of cell cycle progression, inducing apoptosis and inhibiting cancer metastases.[24] It has been reported that β-sitosterol, the predominant phytosterol in plant foods, can inhibit various cancer cells, such as colon T-29, prostate LNCaP, PC-3, DU145, and MDA-MB-23 breast cancer cells.[23–27] Phytosterols are absorbed from the diet in small but significant amounts. Consumption of 1.5–2.0 g of phytosterols per day reduces LDL cholesterol levels by 10–15% over a period of 3 weeks in hyperlipidaemic populations.[24,28] The recently updated US Code of Federal Regulations also states that foods containing at least 0.65 g per serving of plant sterol esters should be eaten twice daily with meals for a total daily intake of at least 1.3 g, as a diet low in saturated fat and cholesterol can reduce the risk of heart disease.[29]

4. Conclusions

Ethanol extract of blackthorn is generally used in folk medicine in Bosnia and Herzegovina as a natural enhancer of erectile function in men, which is also related to the proper function of the prostate. In order to prove the presence of phytosterols in ethanol extracts, considering their positive effect on prostate function, the goal was to validate a method that can detect phytosterols in *P. spinosa* and clarify its use in folk medicine. A simple, specific, precise, fast and reproducible HPLC method was developed for the quantification of phytosterols, relevant marker compounds, in ethanol extracts of *P. spinosa*. The method showed a good linear relationship between peak area and concentrations, acceptable reproducibility and high accuracy. The validation procedure confirms that this method is suitable for the qualitative and quantitative evaluation of the main phytosterols in ethanol extracts of *P. spinosa*, with a good separation of the components of interest. As for the best of our knowledge, there is no available literature data related to the determination of the presence of phytosterols in crude ethanol extracts of blackthorn by a validated HPLC method, so in our case we cannot rely on literature values specifically for this plant species. The analysis of the content of β-sitosterol and accompanying phytosterols in this plant species provides insight into the proven medicinal properties of this plant, its use in phytotherapy, and confirms its traditional use to alleviate the effects of benign prostatic hyperplasia.

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Povzetek

Cilj te raziskave je bil razvoj hitre metode za HPLC separacijo stigmasterola, kampesterola in β-sitosterola v ekstraktih sadežev *Prunus spinosa* L. (črni trn). Vzorce smo pripravili s Soxhletovo ekstrakcijo in ločili na C18 koloni z mobilno fazo iz acetonitrila in metanola ter z uporabo detektorja na diodni niz (PDA). Z optimizirano metodo smo pridobili linearno umeritveno krivuljo v območju 1,70–130 μg mL^{-1} za vse tri fitosterole. Analiza eksternih standardov fitosterolov je pokazala dobro linearnost (R^2 od 0,998 do 0,999); LOD pa smo določili kot 0,32–9,30 μg mL^{-1} ter LOQ 0,98–28,1 μg mL^{-1}. Določitev ponovljivosti in obnovljivosti je pokazala sprejemljive vrednosti %RSD. β-sitosterol je bil prevladujoči fitosterol (51,53–81,03 % od skupnega) v vseh vzorcih. Parametri validacije metode so pokazali, da se lahko to analizno metodo uporabi za točno in natančno določitev kampesterola, stigmasterola in β-sitosterola v izbranih ekstraktih.