

## Qualitative and quantitative HPLC-ELSD-ESI-MS analysis of steroidal saponins in fenugreek seed

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Fenugreek seeds are known as a source of various compounds, the most common of which are steroidal saponins. However, despite the growing interest in this plant material as a healing agent, spice and dietary supplement ingredient, the composition of Polish fenugreek seeds remains unknown. Therefore, the steroidal saponin complex in the seeds of *T. foenum-graecum* cultivated in Poland was qualitatively and quantitatively analyzed by the HPLC-ELSD-ESI-MS method. Two C-18 columns connected in series were used for the first time in analysis of fenugreek saponins and ELS detector parameters were optimized. A total of 26 furostanol saponins were revealed, of which 24 were tentatively identified. The HPLC-ELSD method developed for quantitative analysis was preliminarily validated and the determined amount of steroidal saponins in Polish fenugreek seeds was 0.14 %.

*Keywords:* fenugreek seeds, saponins, HPLC-ELSD-ESI-MS, qualitative and quantitative analysis

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Fenugreek (*Trigonella foenum-graecum*, Fabaceae) is an annual, herbaceous plant, originating from the Mediterranean region and cultivated all over Europe. Fenugreek seeds are known to contain several groups of secondary metabolites, of which, besides flavones (1, 2), isoflavones (1) and polysaccharides (3), the most abundant are steroidal saponins (2, 4–12). The complex of steroidal saponins in fenugreek seeds is composed of different spirostanol and furostanol saponins with straight or branched sugar chains (2, 4–12). The main aglycones are diosgenin and its 25-epimer yamogenin (6). Research on saponins occurring in *Trigonella foenum-graecum* seeds was the subject of many independent studies (2, 4–12) and, as a result, different common names have been given to compounds of the same chemical structure, e.g., protodioscin/compound C, protoneodioscin/trigonelloside C (2, 4, 6–9, 11, 13–16).

Most fenugreek saponins were isolated and structurally recognized by spectroscopic methods (7–9, 11, 13–16); however, some of them were identified with the aid of hyphenated

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techniques like HPLC-MS/MS (2, 4, 6). HPLC equipped with a light diffusion detector was applied to fenugreek saponin analysis only by Petit *et al.* (17), though it is the method of choice in steroidal saponin separation in plant matrices (18–20). Protodioscin was described as a major saponin of fenugreek seeds (21, 22). Steroidal saponins are responsible or co-responsible for several pharmacological effects of fenugreek seeds, including antidiabetic, hypolipidemic, anti-inflammatory and anticancer effects (14, 23, 24). Thus far, most studies have analyzed saponins in the fenugreek seeds of Asian origin. As *T. foenum-graecum* seeds are now becoming increasingly popular as a healing agent and a component of various dietary supplements, their chemical composition must be known because it determines the efficacy and safety of their use. The aim of this work was to analyze qualitatively and quantitatively steroidal saponins in the seeds of *T. foenum-graecum* from Poland using the HPLC-ELSD-ESI-MS method.

## EXPERIMENTAL

### *Materials and reagents*

LC-MS grade acetonitrile and analytical grade formic acid were purchased from Merck (Germany). Water from a Millipore system (Merck) was used in all experiments. The protodioscin standard was purchased from Sigma-Aldrich (USA).

### *Plant material and sample preparation*

Fenugreek (*Trigonella foenum-graecum*) seeds obtained from the herbal company FZL (Kruszynek, Poland) were cultivated in central Poland, collected when mature and dried. Five grams (5.0 g) of dried fenugreek seeds were ground in an electric grinder and extracted with 70 % methanol (2 × 3 h, 2 × 100 mL) at 60 °C using a magnetic stirrer. The obtained extract was lyophilized, dissolved in methanol (1:1) and filtered through a 0.22- $\mu$ m nylon syringe filter (ChemLand, Poland) before HPLC analysis.

### *HPLC systems*

The HPLC system (Shimadzu, Japan) consisted of two LC-20AD pumps, degasser DGU-20A5, semi-micro mixer (100 mL for HPLC-ESI-MS and 0.5–2.6 mL for HPLC-ELSD), controller CBM-20A, thermostat CTO-20AC, autosampler SIL 20AC<sub>XR</sub>, nitrogen generator (Peak Scientific, UK), LCMS-2020 mass spectrometer with ESI ionization and evaporative light scattering detector ELSD 3300 (Alltech Associates, USA). Data was acquired and processed by LabSolution software (version 1.2).

The mobile phase consisted of A – water: formic acid (99.9:0.1, V/V) and B – acetonitrile: formic acid (99.9:0.1, V/V). Separation was performed first on a Discovery C-18 column (150 mm × 2.1 mm, 3  $\mu$ m) according to gradient program I: 0 min – 20 % B, 27 min – 33.5 % B, 45 min – 100 % B. Then, saponins were separated on two Discovery C-18 columns (150 mm × 2.1 mm, 3  $\mu$ m, each) connected in series according to gradient program II: 0 min – 20 % B, 54 min – 33.5 % B, 90 min – 100 % B. Column temperature was 20 °C and the flow rate was 0.2 mL min<sup>-1</sup>. Injection volume was 1  $\mu$ L.

### HPLC-ELSD conditions

ELS detector parameters were experimentally assorted and optimized in the following range: evaporator temperature 40–50 °C, signal strength 4–8, nebulizing gas (N<sub>2</sub>) flow 1.3–2.0 L min<sup>-1</sup>.

### ESI-MS conditions

Mass spectra were acquired using a Shimadzu LCMS 2020 in positive (PI) and negative (NI) ion modes. A full-scan (range *m/z* 800–1600) and SIM (selected ion monitoring) technique for monitoring specific signals was used. The MS detector parameters were: ESI voltage 4.5 kV, nebulizing gas (N<sub>2</sub>) flow 1.5 L min<sup>-1</sup>, desolvation line and block temperatures were: 250 and 200 °C, resp., and drying gas flow (N<sub>2</sub>) was 8.5 L min<sup>-1</sup>. Detector voltage was 2 kV and drying gas flow (N<sub>2</sub>) was 10 L min<sup>-1</sup>.

### Method validation

The method developed for the purpose of quantitative analysis was preliminarily validated in terms of specificity, calibration/linearity, limit of detection (*LOD*), limit of quantitation (*LOQ*), and intra-day and inter-day precision. The data are given in Table I.

Identity of protodioscin in the sample was proven by comparison of the retention time of peak **18** (*t<sub>R</sub>* 36.98 min) and that of the reference substance (*t<sub>R</sub>* 36.98 min), as well as by comparison of the obtained ESI-MS spectrum data for peak **18** and the reference substance. Total amount of saponins was expressed as protodioscin and a calibration curve was constructed for protodioscin. Stock solution of protodioscin in methanol (1 mg mL<sup>-1</sup>) was diluted with methanol and the regression curve was determined on the basis of the analysis of the plot of the peak area for protodioscin (75, 120, 150, 240, 300, 600, 750, 200, 1500, 2400 µg mL<sup>-1</sup>). *LOD* and *LOQ* were determined as the concentration of the standard compound equaling 3× and 10× of the signal-to-noise ratio, resp.

Intra-day precision was assessed by analyzing continuous injections of the same sample (0.24 mg mL<sup>-1</sup> of protodioscin) within one day (*n* = 5), while the inter-day precision was evaluated by analyzing injections of the same sample (0.24 mg mL<sup>-1</sup> of protodioscin) for five consecutive days (*n* = 5 per day). Intra- and inter-day precision were expressed as RSD.

Table I. Validation parameters of the established HPLC-ELSD method for determination of steroidal saponins in fenugreek seeds

Regression equation	$y = x^{0.586726}$ on equat
Coefficient of determination	$R^2 = 0.999$
<i>LOD</i> (µg mL <sup>-1</sup> )	48.99
<i>LOQ</i> (µg mL <sup>-1</sup> )	146.97
Inter-day precision (RSD, %)	1.92
Intra-day precision (RSD, %)	1.80

## RESULTS AND DISCUSSION

### HPLC-ELSD-ESI-MS separation

Chromatographic analysis of steroidal saponins in fenugreek seeds with standard analytical tools is laborious, not only due to their complicated chemical structure and the presence of usually more than two isomers (including 25-epimers) of the same molecular mass, but also due to the lack of commercially available reference substances (with the exception of protodioscin). The detector routinely applied in the HPLC analysis of saponins is ELSD (18–20) since it is known to be universal and sensitive, while the use of a UV detector is inadvisable due to the lack of chromophores in saponin molecules. However, some authors observed separation of saponins by employing UV detection at low wavelengths in a range of 200–210 nm (25, 26).

Use of HPLC hyphenated with detectors MS and ELSD has been so far reported for identification of saponins in the fenugreek seeds of Asian origin (UPLC-ESI-QTOF-MS<sup>n</sup>) (2, 4, 6) and those obtained from an experimental cultivar in France (HPLC-LDD) (17). Using LC coupled with a light diffusion detector on a Kromasil RP-18 column (250 × 4.6 mm,

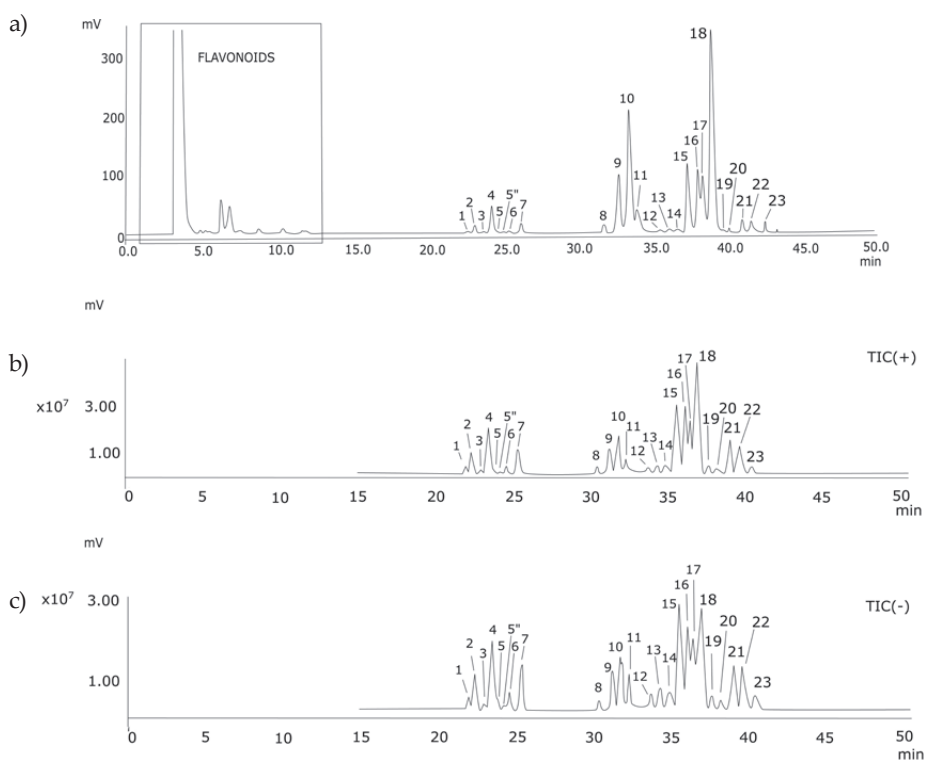


Fig. 1. HPLC-ELSD-ESI-MS chromatograms of steroidal saponins from fenugreek seeds of Polish origin: a) HPLC-ELSD and b) and c) HPLC-ESI-MS [Discovery C-18 (2 × 150 mm × 2.1 mm, 3 μm) columns, gradient elution, program II].

5  $\mu\text{m}$ ), Petit *et al.* (17) obtained separation of fenugreek saponins in the form of 4 peaks in 30 min.

On the basis of literature data (6, 27), the mobile phase used in our experiments was water/acetonitrile to eliminate the formation of methoxy C-22 derivatives of furostanol saponins with a free hydroxyl group at this position. Moreover, Kang *et al.* (6) analyzed the concentration of formic acid in the mobile phase and observed the best shape of saponin peaks at a concentration of 0.1 %. Therefore, the final mobile phase in our experiments was water/acetonitrile with addition of 0.1 % formic acid. Gradient program started from the initial 20 % concentration of acetonitrile/formic acid (99.9:0.1, V/V) in water and the profile of gradient elution as well as the parameters of ELS detector (evaporator temperature, signal strength and nebulizing gas flow rate) were established experimentally. The best ELS detection was achieved at 40 °C of the evaporator, signal strength 4 and nebulizing gas ( $\text{N}_2$ ) flow rate of 1.8 L  $\text{min}^{-1}$ .

HPLC separation of steroidal saponins is usually performed in RP systems, on C-18, C-8,  $\text{NH}_2$  or diol columns (25, 27, 28). In our experiments, the HPLC-ELSD separation was initially performed on a single Discovery C-18 column under gradient elution, according to program I (data not shown). Use of two Discovery C-18 columns ( $2 \times 150 \times 2.1$  mm, 3  $\mu\text{m}$ ) connected in series increased the migration distance of analyzed compounds and, after optimization of gradient elution program (program II), led to their better separation (Fig. 1). HPLC separation with the use of two columns connected in series was applied for the analysis of fenugreek saponins for the first time. As a result, separation of saponins as 24 peaks was achieved. ESI-MS analyses were performed in both positive (PI) and negative (NI) ion modes and the SIM technique was used.

### *Identification of steroidal saponins*

According to literature data (6), steroid saponins under ESI ionization in positive ion (PI) mode form adducts with  $\text{Na}$  ( $\text{M}+\text{Na}$ )<sup>+</sup> and ( $\text{M}+\text{H}-\text{H}_2\text{O}$ )<sup>+</sup> ions, while in negative ion (NI) mode deprotonated molecular ions ( $\text{M}-\text{H}$ )<sup>-</sup> and adducts with formic acid ( $\text{M}-\text{H}+\text{HCOO}$ )<sup>-</sup> were detected. Recognition of the steroid saponin complex in fenugreek seeds of Polish origin was possible by comparing the obtained spectral data ( $m/z$  values of molecular ions and various types of adducts) and chromatographic data (values of  $t_{\text{R}}$ , elution order) with literature data (4–12). As a result, the presence of 26 steroidal saponins was disclosed, of which 24 (1–18, 21–23) were assigned structures of compounds previously identified in the fenugreek seeds of Asian and African origin (2, 4, 6–10). Compound/peak 18 was confirmed to be protodioscin upon  $t_{\text{R}}$  compared to that of the reference substance (36.98 min in both cases), as well as by comparison of the obtained ESI-MS data for peak 18 and the reference substance. Although the applied analytical tool enabled recognition of 26 compounds in fenugreek seeds, it was impossible to identify unseparated 25R and 25S epimers. The obtained chromatographic and spectral data and tentative identification of steroidal saponins in Polish plant material are presented in the Table II.

In the ESI mass spectrum of compound 19, obtained in PI mode, two types of signals were present: at  $m/z$  1073, being an adduct with sodium ( $\text{M}+\text{Na}$ )<sup>+</sup>, and at  $m/z$  1033 corresponding to the ( $\text{M}+\text{H}-\text{H}_2\text{O}$ )<sup>+</sup> ion. In NI mode, compound 19 produced a deprotonated molecular ion at  $m/z$  1049 ( $\text{M}-\text{H}$ )<sup>-</sup> and an adduct with formic acid at  $m/z$  1095 ( $\text{M}-\text{H}+\text{HCOO}$ )<sup>-</sup>. Compound 20 in PI mode gave ions at  $m/z$  923, corresponding to the adduct with sodium

Table II. Chromatographic, spectral data and identified steroidal saponins in the methanol extract from fenugreek seeds of Polish origin

Peak	$t_R$ (min)	(M+Na) <sup>+</sup> $m/z$	(M+H-H <sub>2</sub> O) $m/z$	(M-H) <sup>-</sup> $m/z$	(M-H+HCOO) <sup>-</sup> $m/z$	Molecular formula	Identification (aglycone - S <sub>sugar chain</sub> )	Reference No.
1	21.95	927	887.50	903.60	949.45	C <sub>44</sub> H <sub>72</sub> O <sub>19</sub>	proto-lilagenin-S1/proto-yuccagenin-S1/ 25(27)-eno-protoneogitogenin-S1	4, 6
2	22.15	929	889.25	905.35	951.50	C <sub>44</sub> H <sub>74</sub> O <sub>19</sub>	proto-neogitogenin-S1/proto-gitogenin-S1 (trigoneoside Ia/trigoneoside Ib)	2, 4, 8
3	22.94	1105.90	1065.30	1081.70	1127.60	C <sub>51</sub> H <sub>86</sub> O <sub>24</sub>	proto-neogitogenin-S3/proto-gitogenin-S3 (trigoneoside XVIIa/trigoneoside XVIIb)	2, 4, 6
4	23.5	929.35	889.30	905.45	951.45	C <sub>44</sub> H <sub>74</sub> O <sub>19</sub>	proto-neogitogenin-S4/proto-gitogenin-S4	4, 7
5	23.75	941.40	901.20	917.05	963.45	C <sub>45</sub> H <sub>74</sub> O <sub>13</sub>	proto-lilagenin-S2/proto-yuccagenin-S2	4, 6
5''	24.10	1235.40	1195.65	1211.45	1257.45	C <sub>57</sub> H <sub>96</sub> O <sub>27</sub>	proto-gitogenin-S5/proto-neogitogenin-S5	4, 6
6	24.37	943.30	903.30	919.35	965.55	C <sub>45</sub> H <sub>74</sub> O <sub>19</sub>	proto-neogitogenin-S2 (trigoneoside Xa)	2, 4, 7
7	25.22	943.95	903.30	919.85	965.70	C <sub>45</sub> H <sub>74</sub> O <sub>19</sub>	proto-gitogenin-S2 (trigoneoside Xb)	2, 4, 7
8	30.34	1541.70	1501.55	1517.75	1563.70	C <sub>68</sub> H <sub>110</sub> O <sub>37</sub>	25(27)-eno-proto-diosgenin-S6 (trigoneoside VI/ proto-sceptrumgenin)-S6	4, 7
9	31.17	1543.30	1503.70	1519.85	1565.75	C <sub>68</sub> H <sub>112</sub> O <sub>37</sub>	proto-yamogenin-S7 (trigoneoside Va)	2, 4, 9
10	31.80	1543.70	1503.50	1519.75	1565.90	C <sub>68</sub> H <sub>112</sub> O <sub>37</sub>	proto-diosgenin-S7 (trigoneoside Vb)	2, 4, 9
11	32.10	1411.65	1371.40	1387.70	1433.50	C <sub>63</sub> H <sub>104</sub> O <sub>33</sub>	proto-diosgenin-S8/S9/proto-yamogenin-S8/S9	6, 10
11''	32.10	1545.0	1505.35	1521.90	1567.80	C <sub>68</sub> H <sub>114</sub> O <sub>37</sub>	proto-tigogenin-S10/proto-neotigogenin-S10	6
12	33.77	1249.20	1209.35	1225.75	1271.85	C <sub>57</sub> H <sub>94</sub> O <sub>28</sub>	proto-yamogenin-S11 (trigoneoside XIIIa)	2, 4, 6, 7
13	34.22	1249.0	1209.50	1225.75	1271.80	C <sub>57</sub> H <sub>94</sub> O <sub>28</sub>	proto-diosgenin-S11 (trigoneoside XIIIb)	2, 4, 6
14	34.89	911.0	871.35	887.55	933.35	C <sub>44</sub> H <sub>72</sub> O <sub>18</sub>	proto-diosgenin-S1/proto-yamogenin-S1/proto- makrantogenin-S1/22-deoksy-trigoneoside IIIa/IIIb	2, 4, 6
15	35.54	1087.35	1047.35	1063.45	1109.55	C <sub>51</sub> H <sub>84</sub> O <sub>2</sub>	proto-yamogenin-S12 (trigoneoside IVa)	4, 9

15''	35.54	913.50	873.30	889.25	935.45	C <sub>44</sub> H <sub>74</sub> O <sub>18</sub>	proto-neotigogenina-S1 (trigoneoside IIa/IIIb)	2, 4, 8
16	36.08	1087.50	1047.35	1063.45	1109.55	C <sub>51</sub> H <sub>84</sub> O <sub>23</sub>	proto-diosgenin-S12 (glycoside F)	2, 4, 6, 9
17	36.24	1071.35	1031.35	1047.55	1093.60	C <sub>51</sub> H <sub>84</sub> O <sub>22</sub>	proto-yamogenin-S13 (trigonelloside C, protoneodi- oscin)	2, 4, 7, 15
18	36.98	1071.45	1031.35	1047.55	1093.90	C <sub>51</sub> H <sub>84</sub> O <sub>22</sub>	proto-diosgenin-S13 (compound C, protodioscin)	4, 6, 9, 14
19	37.60	1073.30	1033.50	1049.60	1095.70		n.i.	
20	38.18	923.35	883.30	899.50	945.45		n.i.	
21	39.00	925.30	885.25	901.45	947.60	C <sub>45</sub> H <sub>74</sub> O <sub>18</sub>	proto-yamogenin-S2 (trigofenoside A)	2, 4, 7, 11
22	39.57	925.65	885.25	901.55	947.40		glycoside D	2, 4, 7, 11
23	40.50	927.25	887.45	903.40	949.45	C <sub>45</sub> H <sub>76</sub> O <sub>18</sub>	proto-neotigogenina-S2/proto-tigogenina-S2 (trigoneoside IIIa/IIIb)	2, 4, 6, 8

n.i. – not identified in fenugreek seeds to date.

Aglycone structures according to refs. 6 and 4.

Sugar chains: S1: Glc(6→1)Xyl

S2: Glc(2→1)Rha

S3: Glc(2→1)Rha[(4→1)Glc]

S4: Glc(4→1)Xyl

S5: Glc [[(2→1)Rha]Rha]Rha[(4→1)Rha]

S6: Glc[(4→1)Glc(3→1)Glc(6→1)Glc]Rha[(4→1)Xyl] (2→1)Rha

S7: Glc[(4→1)Glc(3→1)Glc(6→1)Glc]Rha[(4→1)Xyl]Rha

S8: Glc[(4→1)Glc(3→1)Glc(6→1)Glc]Rha

S9: Glc [(2→1)Rha]Rha[(4→1)Glc(3→1)Glc(6→1)Glc]

S10: Glc[(4→1)Glc(3→1)Glc]Rha[(6→1)Glc(4→1)Xyl] (2→1)Rha

S11: Glc[(4→1)Glc(3→1)Glc]Rha

S12: Glc[(4→1)Glc]Rha

S13: Glc[(4→1)Rha]Rha

(M+Na)<sup>+</sup> and at *m/z* 883 being the (M+H-H<sub>2</sub>O)<sup>+</sup> ion. In NI, two signals were abundant: at *m/z* 899 [deprotonated molecular ion (M-H)<sup>-</sup>] and at *m/z* 945 [adduct with formic acid (M-H+HCOO)<sup>-</sup>]. The data obtained for compounds **19** and **20** did not correspond to any of the steroidal saponins previously identified in fenugreek seeds (1–18, 21–23). However, the compound demonstrating signals at the same *m/z* values as those obtained for compound **19** was revealed in HPLC-ESI-MS analysis of *Ruscus* tubers and leaves and was identified as (25*R*)-25,27-dihydroruscoside (**29**).

Yoshikawa *et al.* (30) isolated from male flowers of *Borassus flabellifer* two isomeric steroidal saponins with the molecular formula C<sub>45</sub>H<sub>72</sub>O<sub>18</sub>, which corresponds to the data obtained for compound **20**. Using high resolution mass spectrometry with fast atom bombardment, the authors showed that both isolated compounds were derivatives of 23 $\alpha$ ,27-dihydroxy-diosgenin and 23 $\alpha$ ,27-dihydroxy-yamogenin with the chain of  $\beta$ -D-glucopyranosyl-(4 $\rightarrow$ 1) $\alpha$ -L-rhamnopyranosyl-(2 $\rightarrow$ 1) $\alpha$ -L-rhamnopyranose at C-3 of aglycone (30). Taking into account the presence of proto-diosgenin and proto-yamogenin glycosides in fenugreek seeds, it seems possible that also the glycosides of their 23 $\alpha$ ,27-dihydroxy-derivatives may appear in this plant material. However, full identification of compounds **19** and **20** requires isolation and structure elucidation.

In the HPLC-ELSD-ESI-MS chromatographic profile of steroidal saponins from Polish fenugreek seeds, the most abundant were protodioscin (compound **18**) and trigoneoside Vb (compound **10**). At lower concentrations, trigoneoside IVa (compound **15**) and its 25*R*-epimer – glucoside F (compound **16**), trigoneoside Va (compound **9**) and protoneodioscin (compound **17**) were present. These compounds are mostly derivatives of diosgenin (compounds **10**, **16**, **18**) and its 25*S*-epimer – yamogenin (compounds **9**, **15**, **17**), being the main source of diosgenin released during acid hydrolysis. It has been reported that 25*S* and 25*R* saponinins mutually epimerize (5, 27, 28); therefore, under acidic conditions, yamogenin may be converted to diosgenin.

Comparing the obtained HPLC profile of fenugreek saponins with some literature data, significant differences were found between plant materials of different geographical origin. For example, in the UPLC-ESI-Q/TOF-MS (low CE full scan) analysis of Chinese fenugreek seeds, the most intensive was the peak of a mixture of trigonelloside C (proto-neodioscin) and an unknown furostanol saponin of molecular formula C<sub>51</sub>H<sub>86</sub>O<sub>23</sub> (2). A slightly lower intensity characterized the peaks of mixtures of trigoneoside Ia with trigoneoside XVIIb and trigoneoside Ib with trigoneoside XVIIa (2). Considering the type of aglycone, it should be noted that in the fenugreek seeds of Chinese origin, besides proto-neodioscin being the derivative of proto-yamogenin, the most abundant saponins are derivatives of proto-neogitogenin (trigoneosides Ia and XVIIa) and proto-gitogenin (trigoneosides Ib and XVIIb) (4), while in Polish plant material these compounds are present only in trace amounts (Fig. 1, Table II). On the other hand, in the Indian fenugreek seeds, protodioscin was recognized by HPTLC as the most abundant compound (22).

### Quantitative analysis

Quantitative analysis of steroidal saponins was performed under the established HPLC-ELSD method conditions as described above. The method was preliminarily validated and the results are presented in Table I. In the studied range of concentrations,



the regression curve showed a non-linear relationship, which is consistent with literature data (31, 32).

The total amount of steroidal saponins identified in Polish fenugreek seeds was 0.14 % on dm basis while the total steroidal saponin content in *T. foenum-graecum* seeds of different genotypes from the Indian National Bank of Genomes, determined by spectrophotometric methods, ranged from 0.92 to 1.68 g per 100 g of dry matter (33). Also, Madhava Naidu *et al.* (34), analyzing fenugreek seeds of Indian origin, showed a steroidal saponin content of 5.12 g per 100 g dm, while the amount of these compounds in Chinese fenugreek seeds determined by Wang *et al.* (2) was 6 % of dry matter. In contrast to these data, the Polish plant material has a relatively low content of steroidal saponins.

## CONCLUSIONS

A HPLC-ELSD-ESI-MS method, comprising separation on two columns connected in series, for qualitative and quantitative analysis of saponins, was established. Polish fenugreek seeds were recognized as containing a relatively small amount of steroidal saponins in comparison with Asian plant material. In addition, it was found that other saponins predominated in the seeds of Polish origin compared to Chinese samples. Also, although in both Polish and Indian fenugreek seeds protodioscin was most abundant, the other dominant compounds (saponins) were different. This paper is the first report on the composition of steroidal saponins of fenugreek seeds of Polish origin. In addition, the established HPLC-ELSD-ESI-MS method may be used for analyses of other plant matrices containing steroidal saponins.

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