HPTLC-Densitometric Method for Determination of Eleutherosides B, E, and E_1 in Different *Eleutherococcus* Species

Łukasz Ciesla[1], Daniel Załuski[2], Helena D. Smolarz[2], Michał Hajnos[3], and **Monika Waksmundzka-Hajnos[1,*]**

[1]Department of Inorganic Chemistry, Medical University of Lublin, 6 Staszica Street, 20-081 Lublin, Poland, [2]Department of Pharmaceutical Botany, Medical University of Lublin, 1 Chodzki Street, 20-093 Lublin, Poland, and [3]Department of Pharmacognosy, Medical University of Lublin, 1 Chodzki Street, 20-093 Lublin, Poland

Abstract

The objective of this communication is to report a new high-performance-thin layer chromatography (HPTLC)-densitometric procedure for the separation and quantitative determination of eleutherosides B, E and E_1 in different *Eleutherococcus* samples. Reversed-phase system is applied to resolve the eleutherosides and substances, that are co-eluting in adsorption chromatography systems. *E. henryi* is found to be the species with the greatest eleutheroside content. Validation of the procedure reveals it is a specific, precise, accurate, stabile and robust chromatographic method. Quantitative HPTLC technique, of the three eleutherosides, in different eleuthero root samples is reported for the first time.

Introduction

The *Eleutherococcus* Maxim. [*Acanthopanax* (Decne. et Planch.) Witte] genus comprises 38 species growing in Eastern Asia, from the Himalayas to Vietnam, and from Northeastern Russia to North Philippines, with 18 of them being a part of the Chinese flora (1,2).

All the *Eleutherococcus* species contain many constituents with wide biological activities, (e.g., eleutherosides, flavonoids, senticoside, triterpenic saponins, vitamins, minerals, essential oil, and complex polysaccharides) isolated from roots and leaves (3). The main chemical substances are eleutherosides. These are the phenol glycosides and triterpenic saponins. *Eleutherococcus* species contain eleutherosides including A, B, C, D, E (4), E_1 (5), F, G (4), I, K, L, and M (6). They have traditionally been used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes. The natural products isolated from *Acanthopanax* species have been shown to have various levels of activity as antibacterial, anticancer, anti-inflammatory, antigout, antihepatitis, antihyperglycemic, antileishmanicidic, antioxidant, antipyretic, antixanthine oxidase, choleretic, radioprotectant, hemostatic, immunostimulatory, and hypocholesterolemic effects (7).

*Author to whom correspondence should be addressed: email monika.hajnos@am.lublin.pl.

Eleutherococcus senticosus is the only pharmacopoeial species. Its monographs can be found in the European Pharmacopoeia (Pharm. Eur) or American Herbal Pharmacopoeia monographs (8,9).

As eleutherosides are thought to be the most pharmacologically active constituents found within *Eleutherococcus* spp., it is important from the standpoint of herbal drug standardization to develop a method for the quantitative determination of these compounds. According to the Pharm. Eur. monograph the root of *E. senticosus* should contain minimum 0.08% for the sum of eleutheroside B and eleutheroside E (8). Several thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) methods have been established to analyze *Eleutherococcus* samples. The majority of these techniques is focused on the fingerprint development, not considering the amount of eleutherosides. In case of the quantitative methods, they are usually focused on assessing the amount of only one eleutheroside (E or B) some present, quantitation of both of them (10–13). However, as far as could be ascertained, there is no method established for the simultaneous determination of the three eleutherosides–B, E, and E_1.

HPLC is the leading chromatographic technique used to quantify the amount of different compounds in complex samples. It is often overlooked that with proper instrumentation and suitable methodology, quantitative determinations by HPTLC could also give reliable results at lower cost (14). HPTLC has been proven to be a suitable technique to perform quantitative analysis of different compounds found within complex natural samples (15–18). As far as eleutheroside determination is considered, an HPTLC method has been developed, for quantitative determination of eleutheroside B, in a liquid extract prepared from seven plants, including *E. senticosus* (19).

The aim of this study was the development of HPTLC-densitometric method to quantify the amount of eleutherosisdes B, E and E_1 in different *Eleutherococcus* species. It is the first time, that a fully validated HPTLC method has been established for quantitative analysis of the three eleutherosides. A reversed-phase (RP) system was applied to resolve the constituents, co-eluting in adsorption chromatography systems. Assessing the amount of these constituents within different *Eleutherococcus* species has not been reported before. As far as it could be deter-

mined, this is the first time that an HPTLC-densitometric method is applied to quantify the amount of three eleutherosisdes in *Eleutherococcus* samples.

Experimental

Apparatus and reagents

The standards of eleutherosides were obtained from ChromaDex (Santa Ana, CA). The structures of eleutherosides E, E_1, and B are presented in Figure 1.

The following solvents and regents were used for performing the experiments: methanol, acetic acid, distilled water, ethanol, and sulfuric acid. They were all of analytical grade, purchased from Polish Reagents (Gliwice, Poland). TLC was performed on 10 × 20 cm glass-backed RP-18 WF_{254S} HPTLC plates from E. Merck (Darmstadt, Germany).

Ethanolic solutions (1 mg/mL) of all standards and plant extracts were applied to chromatographic plates bandwise, by means of a Camag automatic TLC sampler (Camag, Muttenz, Switzerland) and developed in horizontal DS chambers (Chromdes, Lublin, Poland). Plates, after derivatization, were documented with the use of Camag TLC Reprostar 3 with computer program Videostore, and scanned with densitometer Camag TLC scanner with computer program CATS 4 (Camag).

Plant materials

Roots of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., *Eleutherococcus setchuensis* (Harms) Nakai, *Eleutherococcus sessiliflorus* (Rupr. & Maxim.) S.Y. Hu, *Eleutherococcus gracilistylus* (W. W. Smith) S.Y. Hu, *Eleutherococcus henryi* Oliv., and *Eleutherococcus divaricatus* (Siebold & Zucc.) S.Y. Hu were collected from arboretum in Rogów (Poland) in October 2008. The plant material was dried and immediately milled according to accepted standard for roots (20). Voucher specimens were deposited at the Department of Pharmaceutical Botany, Medical University of Lublin, Poland.

Extraction conditions

Each sample (~15 g) was soaked in 150 mL 75% ethanol, in a round-bottomed flask, for 24 h. After that the samples were sonicated in an ultrasonic bath (Polsonic, Warsaw, Poland) at room temperature, for 15 min. Liquids were carefully filtered and the plant material was re-extracted for 15 min with 100 mL of the same solvent. The obtained extracts were combined and filled with ethanol up to a volume of 350 mL. Fourteen milliliters of each extract was taken and concentrated to a volume of 12 mL, in nitrogen stream.

Sample clean-up

Samples were cleaned on Bakerbond SPE Octadecyl C_{18} microcolumns (500 mg, 3 mL; J.T. Baker, Phillipspurg, NJ) previously activated with 2 mL 99.8% ethanol, then 2 mL distilled water and finally 2 mL 75% ethanol.

Twelve milliliters of each extract was filtrated through the columns under reduced pressure. Each column was washed then with 4 mL 75% ethanol. The obtained 16 mL of each sample was concentrated, in nitrogen stream, to a volume of 0.6 mL. The prepared samples underwent chromatographic analysis. To confirm the complete elution of the eleutherosides from SPE microcolumns, they were additionally eluted with 10 mL of 75% ethanol. The eluate was evaporated, the residue dissolved in 0.2 mL ethanol and applied, with the use of automatic sampler, onto an HPTLC plate and chromatographed with solvent system: methanol–distilled water–acetic acid (3:6:1, v/v/v).

Thin-layer chromatography

Standard and sample solutions were applied bandwise (band length 6 mm, band velocity 10 mm/s, track distance 12 mm, distance from left edge 30 mm and from low edge 8 mm) to the chromatographic plates. The standard solutions were applied, in the following amounts: 1 µL, 2 µL, 3 µL, 5 µL, 7 µL, 10 µL, 12 µL, 15 µL, and 20 µL, to prepare calibration curves. They were all applied in triplicate. In case of the *Eleutherococcus* samples, 5.0 µL was applied to chromatoplates.

The plates were developed in horizontal DS chambers, at ambient temperature 20 ± 1°C. The following mobile phase was used: methanol–distilled water–acetic acid (6:3:1, v/v/v). The plates were developed to distance of 90 mm. The plates were dried at room temperature for 20 min before derivatization.

Derivatization and densitometrical scanning

The plates were derivatized with the use of Liebermann-Burchard reagent. To prepare it, 5 mL sulfuric acid was carefully added to 5 mL acetic anhydride, the mixture was then mixed with 40 mL 95% ethanol. All the chemicals, used for the reagent preparation, were ice-cold. The plates were immersed in reagent for 1 s then heated at 105°C for 10 min.

The pictures of plates were taken in visible and UV light (λ = 366 nm) and scanned at different wavelengths. UV spectra of

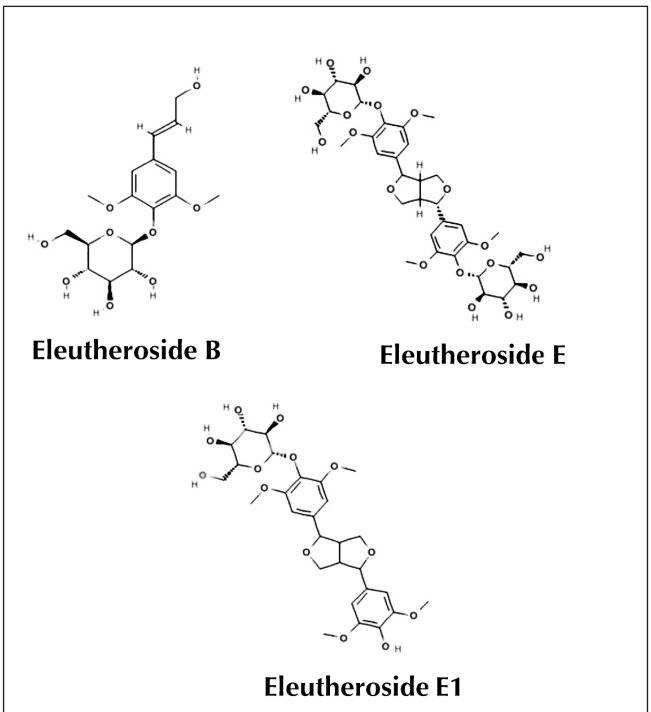

Figure 1. Chemical structures of the quantified eleutherosides.

eleutherosides, identified in the extracts, were also taken and compared with those obtained for standards (see Figure 2). The plates were densitometrically scanned with slit dimension 1 mm × 0.1 mm. After the development, bands in the extracts were identified by matching their R_F values and UV spectra, with those obtained for standards. The purity of the peaks, in all analyzed samples, was checked by taking the spectra at peaks' centre and flanks.

Method validation

The method has been validated in the following parameters: specificity, linearity, precision, accuracy, robustness, and stability. Limit of detection (LOD) and limit of quantitation (LOQ) values were also determined.

As far as specificity was concerned, the obtained videoscans and densitograms were compared. Spectral evaluation of the reference substances and the resolved components, from the extracts, was performed to check whether there is co-elution of compounds.

Linearity was determined by applying standard solutions of the eleutherosides in the range 1–20 µg per band. The linearity was determined by plotting peak areas against concentration in the aforementioned range and checking the correlation coefficient.

To assess the precision of the method, RSD (%) values were estimated for each eleutheroside. The data were obtained from three applications of the sample across the entire plate.

The accuracy was assessed in a recovery experiment. A 5.0 µL *E. senticosus* sample was spiked with known amount of all the eleutheroside standards: 1 µg, 2 µg, and 3 µg. Recovery was calculated as quotient of measured amount and added amount of analyte.

The LOD and the LOQ were calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approaching the LOD according to the formulas: LOD = 3.3 (SD/S) and LOQ = 10 (SD/S). The standard deviation of the response was determined based on the standard deviation of y-intercepts of regression lines (21).

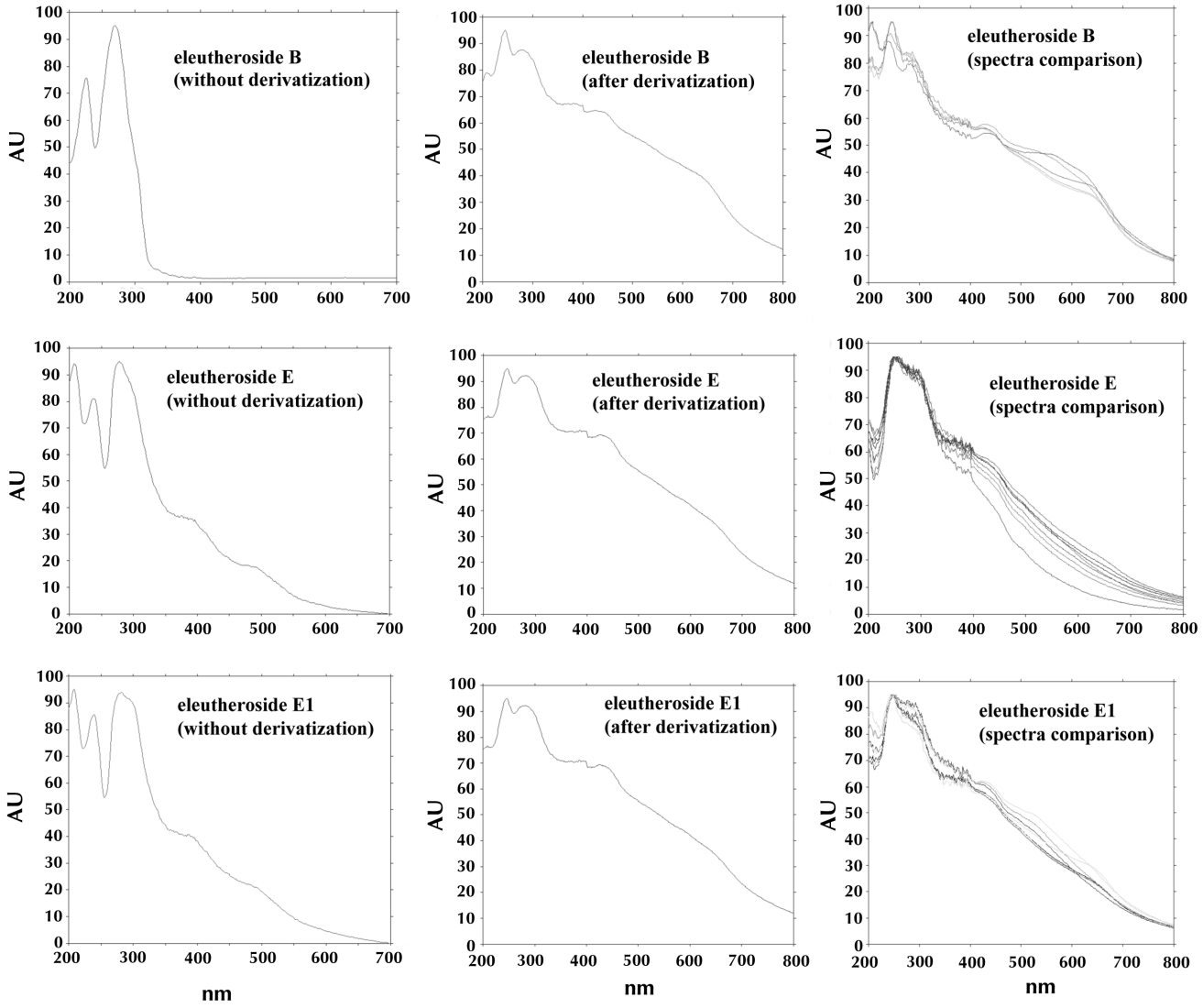

Figure 2. UV spectra of the investigated compounds taken before and after derivatization. The UV spectra of standards were compared with those obtained after separation of the analyzed eleuthero samples (spectra comparison).

Stability of the sample during the chromatographic processes was performed by means of 2D-TLC method. The analyzed samples were applied as spots, each sample on separate plate, and developed in two, perpendicular directions with the same eluent: methanol–distilled water–acetic acid (6:3:1, v/v/v). For checking the stability of the sample, after the application, the sample of *E. senticosus* was applied at 1, 2, 3 h, and just before the development. The obtained chromatograms were compared.

As far as method robustness is considered, the following conditions were checked: drying time, mobile phase composition, developing distance, heating temperature, time between derivatization and densitometrical scanning. The plate was derivatized after 15, 20, 30, and 40 min after development. For checking the influence of mobile phase composition on the final results, mobile phases with minor changes, in their solvent content, were checked. The following developing distances were checked: 70, 80, and 90 mm.

The plates were heated, after derivatization, for 1, 2, 5, 10, and 15 min. The plates were densitometrically scanned soon after the completion of derivatization, as well as after 10, 20, 30, and 60 min. The areas under the common peaks were compared.

Results and Discussion

The best resolution of eleutherosides, found in different *Eleutherococcus* root samples, was obtained on RP-18 W plates with the application of the following solvent system: methanol–water–acetic acid (6:3:1, v/v/v). Chromatographic analysis was preceded by a clean-up procedure, on C_{18} microcolumns, to purify the sample. Components, found within the purified extracts, were easier to separate, as their amount was lesser when compared to non-purified samples. To check whether the eleutherosides were completely eluted from the SPE microcolumns, the cartridges were flushed with 10 mL 75% ethanol. The HPTLC-densitometric examination of the eluate, showed the absence of bands characteristic for the investigated eleutherosides. It confirmed the complete yield of eleutherosides from the microcolumns, according to the proposed method.

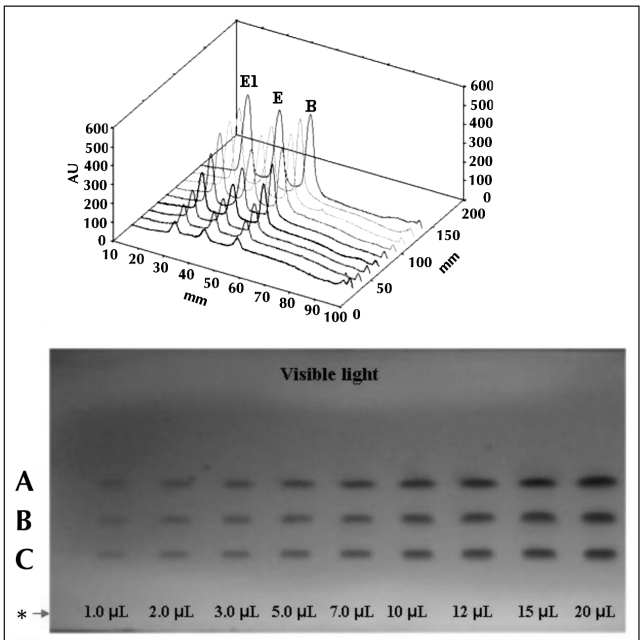

Figure 3. Videoscan and densitogram presenting the resolution of the three eleutherosides, on RP-18 W plate, with the use of solvent system: ethanol–distilled water–acetic acid (6:3:1, v/v/v). The plate was immersed into Liebermann-Burchard reagent and documented soon after the completion of derivatization. A = eleutheroside B; B = eleutheroside E; and C= eleutheroside E1. * is the application position.

As it is apparent from Figure 3, all the eleutheroside standards were separated on baseline. In the applied chromatographic system eleutherosides are characterized by the following R_F values: eleutheroside B – 0.58, eleutheroside E – 0.47, and eleutheroside E_1 – 0.33.

Liebermann-Burchard reagent was used to derivatize the chromatograms. Two modes of derivatization were checked: spraying and dipping. More reproducible results were obtained in the latter case.

As the amount of eleutherosides was established after derivatization, thus the UV spectra of eleutherosides B, E, and E_1 were taken after dipping in Liebermann-Burchard reagent. As can be seen in Figure 2, the spectra of the compounds, taken before and after derivatization, differ for all the three elutherosides. The plates were scanned at the observed λ_{max}, that were the same for the analyzed compounds: 250 nm, 295 nm, and 430 nm. The best results were obtained at 250 nm.

Calibration plots of average peak areas (the standards were applied in triplicate) against substance quantity were constructed for each standard separately. The obtained quantitative calibration data were best fitted with polynomial regression (the Kubelka and Munk theory). Polynomial regression gave the following correlation coefficient (R^2) values for the standards, in the range 1 µg–20 µg: B – 0.9979, E – 0.998, E_1 – 0.9989. However, a pseudo-linear range, 1 µg–10 µg, was observed for the three eleutherosides, where linear regression gave satisfactory results (see Table I).

The optimized chromatographic procedure was applied to quantify the amount of eleutherosides B, E, and E_1 in different *Eleuthrococcus* samples. The obtained results, along with precision values, are presented in Table II. From these data it is apparent that in the majority of the analyzed samples eleutheroside B constituted the greatest part of eleutheroside content. The highest concentration of eleutheroside B was detected in *E. henryi* (3.42 mg/g). In *E. setchuensis, E. divaricatus*, and *E. gracilistylus* the content of this component ranged between 1.28 and 1.8 mg/g. Roots of *E. senticosus* and *E. divaricatus* are char-

Table I. Calibration Curves of the Eleutherosides and their LOD and LOQ Values

Eleutheroside	Slope	Intercept	R^2	LOD (µg/band)	LOQ (µg/band)
B	727.69 ± 23.53	2210.4 ± 159.14	0.9979	0.72	2.17
E	830.79 ± 15.89	1692.9 ± 93.3	0.9993	0.37	1.12
E1	917.27 ± 9.72	1589.2 ± 59.42	0.9997	0.21	0.65

acterized by the lowest eleutheroside E content. Both eleutheroside E and eleutheroside E_1 are present in all the studied species, but in a smaller amount than eleutheroside B. Interestingly, a large content of the sum of eleuthetoside B and E was found in *E. henryi* (4.66 mg/g), whereas *E. senticosus,* the only pharmacopoeial species, is characterized by the lowest amount of eleutherosides B and E (1.29 mg/g) from among six studied species.

The method was validated according to the commonly accepted procedures for quantitative HPTLC methods (14). As it is apparent from Figures 4 and 5 all the eleutherosides are well resolved from other constituents. The comparison of the UV spectra, for the identified compounds, and the standards confirms there is no compound co-elution (Figure 2). The linear regression was obtained in the range of 1 µg–10 µg per band, that is satisfactory to assess the amount of eleutherosides within the analyzed samples. The obtained precision values are good and satisfactory, and meets the criteria for quantitative TLC method. The presented method is also characterized by good accuracy values, determined in the recovery test (see Table III).

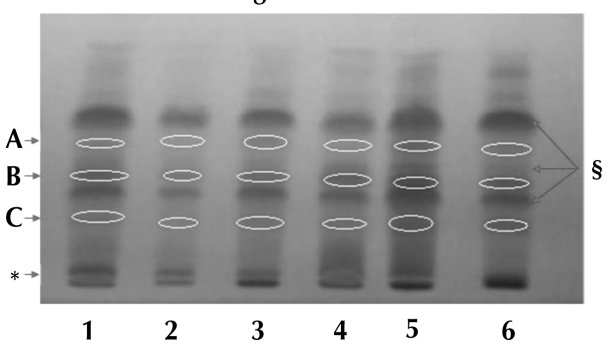

Figure 4. Videoscan presenting six eleuthero root samples developed on RP-18 W plate with solvent system: methanol–distilled water–acetic acid (6:3:1, v/v/v). The plate was immersed into Liebermann-Burchard reagent and documented soon after the completion of derivatization. A = eleutheroside B; B = eleutheroside E; and C= eleutheroside E1. 1 = *E. setchuensis;* 2 = *E. senticosus;* 3 = *E. divaricatus;* 4 = *E. gracilistylus;* 5 = *E. henryi;* 6 = *E. sessiliflorus.* * is the application position, and § indicates substances that coeluted in the NP system

Table II. Eleutheroside Content in Extracts Obtained from Different Eleutherococcus spp Expressed in mg per 1 g of the Sample Dry Weight

Eleutheroside	*E. setchuensis*	*E. senticosus*	*E. divaricatus*	*E. gracilistylus*	*E. henryi*	*E. sessiliflorus*
B (mg/g)	1.8	0.77	1.6	1.28	3.42	0.55
(%)	0.18	0.08	0.16	0.13	0.34	0.05
SD	0.08	0.07	0.05	0.05	0.08	0.04
RSD	4.44	9.09	3.13	3.91	2.34	7.27
E (mg/g)	1.13	0.52	0.92	1.3	1.24	1.05
(%)	0.11	0.05	0.09	0.13	0.12	0.1
SD	0.04	0.04	0.05	0.03	0.05	0.03
RSD	3.54	7.69	5.43	2.31	4.03	2.86
E1 (mg/g)	0.32	0.23	0.90	0.42	0.86	0.62
(%)	0.03	0.02	0.09	0.04	0.09	0.06
SD	0.03	0.01	0.03	0.02	0.02	0.04
RSD	9.38	4.35	3.33	4.76	2.33	6.45

The result obtained in 2D-TLC test indicates the stability of the sample during the chromatographic process. All the spots were placed on the plate's diagonal, there are no artifact formation nor chemisorption during chromatography on RP-18 W plates, with the proposed solvent system. The sample components are also stable after the application to the chromatoplate, as chromatograms obtained for extracts applied 1, 2, 3 h, and just before the development, were identical.

All the experimental conditions, checked in the robustness test, had minor influence on the final results, except for the heating temperature, heating time and time between derivatization and densitometrical scanning. When the temperature was lower than 100°C, zones were fainter, the values of the surfaces, under the common peaks, were also lesser than in the optimized procedure. The best results are obtained, when the plate is heated for 10 min after immersion. During method development, it is important to scan the plates soon after completion of derivatization, as the derivatized zones are not stable. After 30 min, significant changes in the surface areas under the peaks, were observed.

As already stated in the Introduction, several TLC methods have been proposed to analyze *Eleutherococcus* species. These methods were performed on TLC or HPTLC silica gel plates, in normal-phase systems. They were all focused on defining the acceptance criteria for *Eleutherococcus senticosus* (fingerprint development). The applicability of these methods for quantitative determination of eleutheroside B, E, and E_1 was verified in this experiment. All the systems, found in the literature, are well suited for identification purposes, however for quantitative analysis further adjustment would be necessary. The main problem is the co-elution of eleutherosides with other substances, seen as blue bands (UV, λ = 366 nm), on silica layers. The co-elution of eleurtheroside B with chlorogenic acid was also reported in the literature (10,22). Stepanov proposed sample purification by column chromatography on an alumina sorbent, as the co-elution of eleutheroside B with phenolic acids and/or flavonoids was observed (19). Experiments performed in the laboratory have shown, that normal-phase systems failed to resolve eleutherosides and the aforementioned co-eluting substances. Thus RP chromatographic systems, on RP-18 W plates, were further checked. The application of the optimized RP system caused the analyzed eleutherosides, as well as the co-eluting compounds, were well resolved, the previously co-eluting substances are marked on the chromatoplate presented in Figure 4. The applied RP system has also additional advantage over those reported in the literature [e.g., TLC methods used for *E. sencticosus* identification in AHP (9), or in Wagner TLC Atlas (23)] – it does not contain toxic chloroform. The mobile phase of the Ph.Eur.5 (8), although avoiding the use of chloroform, does not provide a chromatogram with sufficient separation of the zones close to eleutheroside B.

Table III. Recovery study for eleutherosides B, E, E1 from *E. senticosus*.

Compound	Eleutheroside in 5.0 µL sample (µg)	Spiking amount (µg)	Theoretical value (µg)	Experimental value (µg)	Recovery (%)
Eleutheroside B				4.72	98.3
	3.80	1.0	4.80	4.69	97.7
				4.75	98.9
				5.65	97.4
	3.80	2.0	5.80	5.58	96.2
				5.70	98.3
				6.73	98.9
	3.80	3.0	6.80	6.70	98.5
				6.68	98.2
Eleutheroside E				3.62	101.4
	2.57	1.0	3.57	3.65	102.2
				3.54	99.2
				4.68	102.4
	2.57	2.0	4.57	4.62	101.1
				4.67	102.2
				5.70	102.3
	2.57	3.0	5.57	5.64	101.3
				5.67	101.8
Eleutheroside E1				2.34	109.9
	1.13	1.0	2.13	2.32	108.9
				2.29	107.5
				3.19	101.2
	1.13	2.0	3.13	3.25	103.8
				3.23	103.2
				4.31	104.4
	1.13	3.0	4.13	4.27	103.4
				4.28	103.6

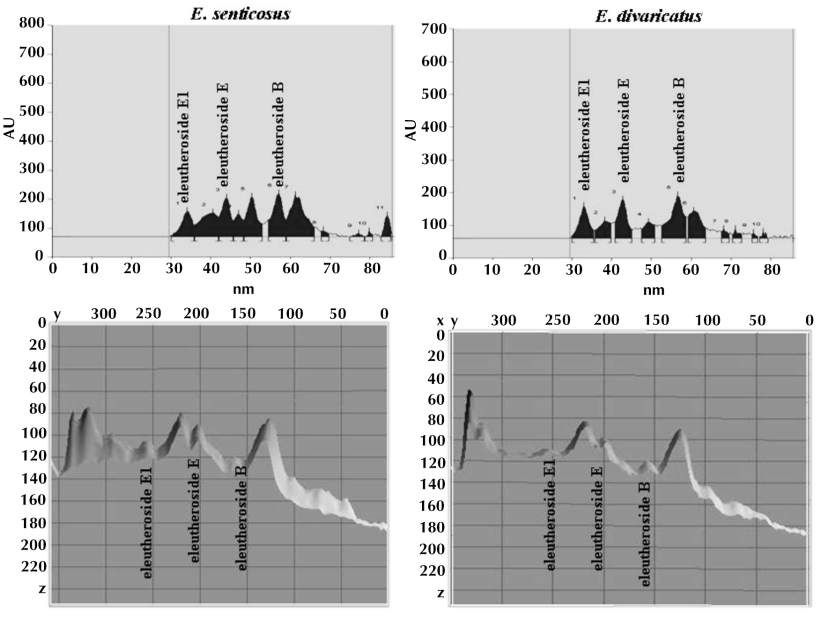

Figure 5. Exemplary densitograms and images, obtained with image processing program (Image J). The densitograms were obtained after scanning the RP18 W plates at λ = 250 nm, with slit dimension 1 mm × 0.1 mm.

Despite bands' diffusion effect, a common HPTLC drawback, which was observed in the applied RP system, it had only a minor influence on the obtained results.

Another problem, that has to be overcome in the analysis of eleutherosides, is their detection. Unfortunately only eleutheroside B is detected under UV light (λ = 254 nm). Literature indicates several derivatizing agents to be suitable for performing derivatization of eleutherosides: anisaldehyde solution (8), antimony chloride (9), vanillin reagent (23), or sulfuric acid reagent (14). The application of the aforementioned reagents yield colorful chromatograms (except for sulfuric acid agent), however they did not enable performing quantitative analysis, mainly due to colored background. Reproducible results were obtained with the application of Liebermann-Burchard reagent.

The optimized chromatographic procedure is well suited for quantitative analysis of eleutherosides in real botanical samples. An interesting conclusion can be derived, from the obtained results: roots of *E. henryi* are the best source of biologically active eleutherosides, especially of eleutheroside E among the studied taxons of *Eleutherococcus*. Thus this species may constitute a good candidate for an ingredient of herbal remedies.

Conclusion

Quantitative HPTLC procedure of eleutherosides in six *Eleutherococcus* species has been described in this paper. The presented method is easy to operate, the results are obtained fast at a relatively low cost. An RP chromatography system was applied to resolve the constituents co-eluting, in normal-phase systems, presented in the literature. An interesting thing to notice is that the pharmacopoeial species *E. senticosus* has the lowest eleutherosides content in comparison to other five investigated species. Thus these species may become an alternative source, of biologically active eleutherosides. The validation procedure revealed that the method meets all the criteria for quantitative HPTLC method. The presented technique may be used for preliminary screening of eleutherosides content in eleuthero root samples.

Acknowledgements

We thank Piotr Banaszczak, the director of Arboretum in Rogów, for provision of eleuthero root samples.

References

1. J. Tumiłowicz and P. Banaszczak. Woody species of Araliaceae at the Rogów Arboretum. *Rocznik Dendrologiczny* **54:** 35–50 (2006).
2. W. Seneta. Drzewa i krzewy liciaste. Vol. 1. PWN, Warsaw, 1991 (in Polish).
3. S. Nishibe, H. Kinoshita, and H. Takeda. Phenolic compounds from stem and bark of Acanthopanax senticosus and their pharmacological stressed rats. *Chem. Pharm. Bull.* **38:** 1763–1765 (1990).
4. A.M. Shohael, H.N. Murthy, and E.J. Hahn. Increased eleutheroside production in Eleutherococcus sessiliflorus embryogenic suspension cultures with methyl jasmonate treatment. *Bioch. Engin. J.* **38:** 270–273 (2008).
5. X. Li, L. Dayna, and A. Ikhlas. A new lignan glycoside from Eleutherococcus senticosus. *Planta Med.* **67:** 776–778 (2001).
6. G.M. Frolova and Y.S. Ovodov. Triterpenoid glucosides of Eleutherococcus senticosus leaves. II. Structure of eleutherosides I, K, L. Khim. *Prir. Soed.* **5:** 618–622 (1971).
7. S. Lee and K.H. Shin. The chemistry of secondary products from Acanthopanax species and their pharmacological activities. *Nat. Prod. Science* **8:** 111–126 (2002).
8. European Pharmacopoeia, 5th ed. Strasbourg, Council of Europe, 2003.
9. AHP monographs: American Herbal Pharmacopoeia and Therapeutic Compendium. Scotts Valley, CA: American Herbal Pharmacopoeia.
10. P.N. Yat, J.T. Arnason, and D.V.C. Awang. An improved extraction procedure for the rapid, quantitative high-performance liquid chromatographic estimation of the main eleutherosides (b and e) in *Eleutherococcus senticosus* (eleuthero). *Phytochem. Anal.* **9:** 291–295 (1998).
11. S.I. Feng, F. Hu, J.X. Zhao, X. Liu, and Y. Li. Determination of eleutheroside E and eleutheroside B in rat plasma and tissue by high-performance liquid chromatography using solid-phase extraction and photodiode array detection. *Eur. J. Pharm. Biopharm.* **62:** 315–320 (2006).
12. J.S. Kang, P.T. Linh, X.F. Cai, H.S. Kim, J.J. Lee, and Y.H. Kim, Quantitative determination of eleutheroside B and E from Acanthopanax species by high performance liquid chromatography. *Arch. Pharm. Res.* **24:** 407–411 (2001).
13. Z. Wang, L. Zhang, and Y. Sun. Semipreparative separation and determination of eleutheroside E in *Acanthopanax giraldii Harms* by high-performance liquid chromatography. *J. Chromatogr. Sci.* **43:** 249–252 (2005).
14. E. Reich and A. Schibli. High-performance thin-layer chromatography for the analysis of medicinal plants. Thieme, New York, 2008.
15. K. Dhalwal, V.M. Shinde, A.G. Namdeo, K.R. Mahadik, and K.S. Shivajirao. Development and validation of a TLC-densitometric method for the simultaneous quantitation of strychnine and brucine from Strychnos spp. and its formulations. *J. Chromatogr. Sci.* **45:** 706–709 (2007).
16. P.C. Dhandhukia and V.R. Thakkar. Separation and quantitation of jasmonic acid using HPTLC. *J. Chromatogr. Sci.* **46:** 320–324 (2008).
17. V. Widmer, D. Handloser, and E. Reich. Quantitative HPTLC analysis of artemisinin in dried *Artemisia annua L.*: A practical approach. *J. Liq. Chromatogr. Rel. Technol.* **30:** 2209–2219 (2007).
18. M. Wójciak-Kosior, T. Krzaczek, G. Matysik, and A. Skalska-Kaminska. HPTLC-densitometric method of determination of oleanolic acid in the Lamii albi flos. *J. Sep. Sci.* **28:** 2139–2143 (2005).
19. A.S. Stepanov. Quantitative determination of eleutheroside in Elima liquid extract by high-performance thin-layer chromatography. *Pharm. Chem. J.* **37:** 217–219 (2003).
20. Polish Pharmacopoeia VI, Polish Pharmaceutical Society, Warsaw 2002.
21. T. Tuzimski and E. Bartosiewicz, Correlation of retention parameters of pesticides in normal and RP systems and their utilization for the separation of a mixture of ten urea herbicides and fungicides by two-dimensional TLC on cyanopropyl-bonded polar stationary phase and two-adsorbent-layer Multi-K plate. *Chromatographia* **58:** 781–788 (2003).
22. I. Slacanin, A. Marston, K. Hostettmann, D. Guedon, and P. Babe. The isolation of Eleutherococcus senticosus constituents by centrifugal partition chromatography and their quantitative determination by high performance liquid chromatography. *Phytochem. Anal.* **2:** 137–142 (1991).
23. H. Wagner and S. Bladt. *Plant Drug Analysis: A Thin Layer Chromatography Atlas.* 2nd edition, Springer, Berlin, 1995.

Manuscript received October 20, 2009;
revision received January 18, 2010.