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An Improved Extraction Procedure for the Rapid, Quantitative High-Performance Liquid Chromatographic Estimation of the Main Eleutherosides (B and E) in *Eleutherococcus senticosus* (Eleuthero)

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Eleutherosides were extracted with aqueous methanol and analysed by reversed-phase high performance liquid chromatography on a C-18 column. Recovery was better than 80% for eleutheroside B within the concentration range 500 to 10,000 μg, and similarly for eleutherosides E in the range 500 to 2,500 μg. Lower recovery values were obtained at higher concentrations for eleutheroside E due to its insolubility in alcoholic solutions. The use of trifluoroacetic acid solution in methanol was found to resolve the solubility problem. The concentration ranges over which a linearity of response for both eleutherosides could be validated were improved significantly by this adjustment. © 1998 John Wiley & Sons, Ltd.

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Keywords: Eleutherosides; Eleuthero; *Eleutherococcus senticosus*; Siberian ginseng; high-performance liquid chromatography.

INTRODUCTION

The need for a convenient and rapid method for screening purported eleuthero products is demanding. This is because of the desirability of identity assurance, and the increasing tendency of manufacturers to claim higher quality formulations standardized for eleutherosides content. The toxicity aspect associated with the consumption of purported eleuthero ("Siberian ginseng") has been emphasized by two medical publications in the past 5 years, attributing androgenization (Awang, 1991) and digoxin serum elevation (Awang, 1996), although some of the products have clearly been misidentified.

Eleutherococcus senticosus, Rupr. & Maxim. (Syn. *Acanthopanax senticosus* Harms) is a shrub found in Russia, Northern China, Korea and Japan, which belongs to the same Araliaceae family as ginseng (*Panax* spp.). The designation (Fulder, 1980; Slacanin *et al.,* 1991; Awang, 1996) "Siberian ginseng" is a commonly applied misnomer, and the more appropriate "eleuthero" is preferred by some experts. The roots and rhizome of the plant have long been used as a stimulant and to enhance overall resistance to disease and stress (Awang, 1996); claimed therapeutic properties that are shared with traditional ginseng. Considerable interest has been generated in the medicinal properties of the alcoholic extracts (Fulder, 1980; Farnsworth *et al.,* 1985; Sprecher, 1989) Most of the research concerning the pharmacological effects of *E. senticosus* was initiated in Russia,

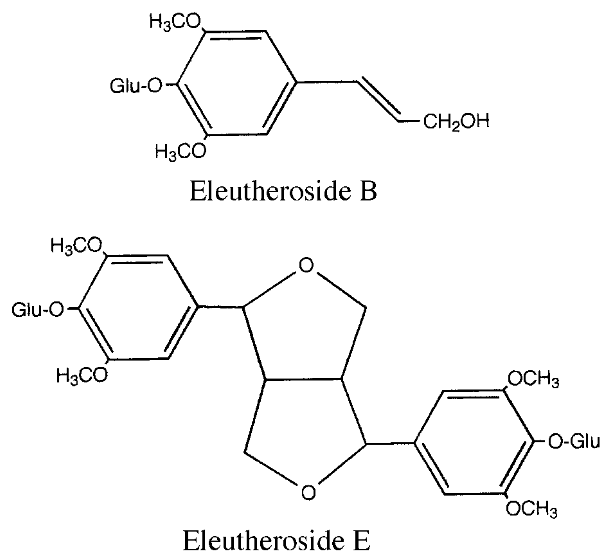

Figure 1. Structures of eleutherosides B and E determined quantitatively in the present study.

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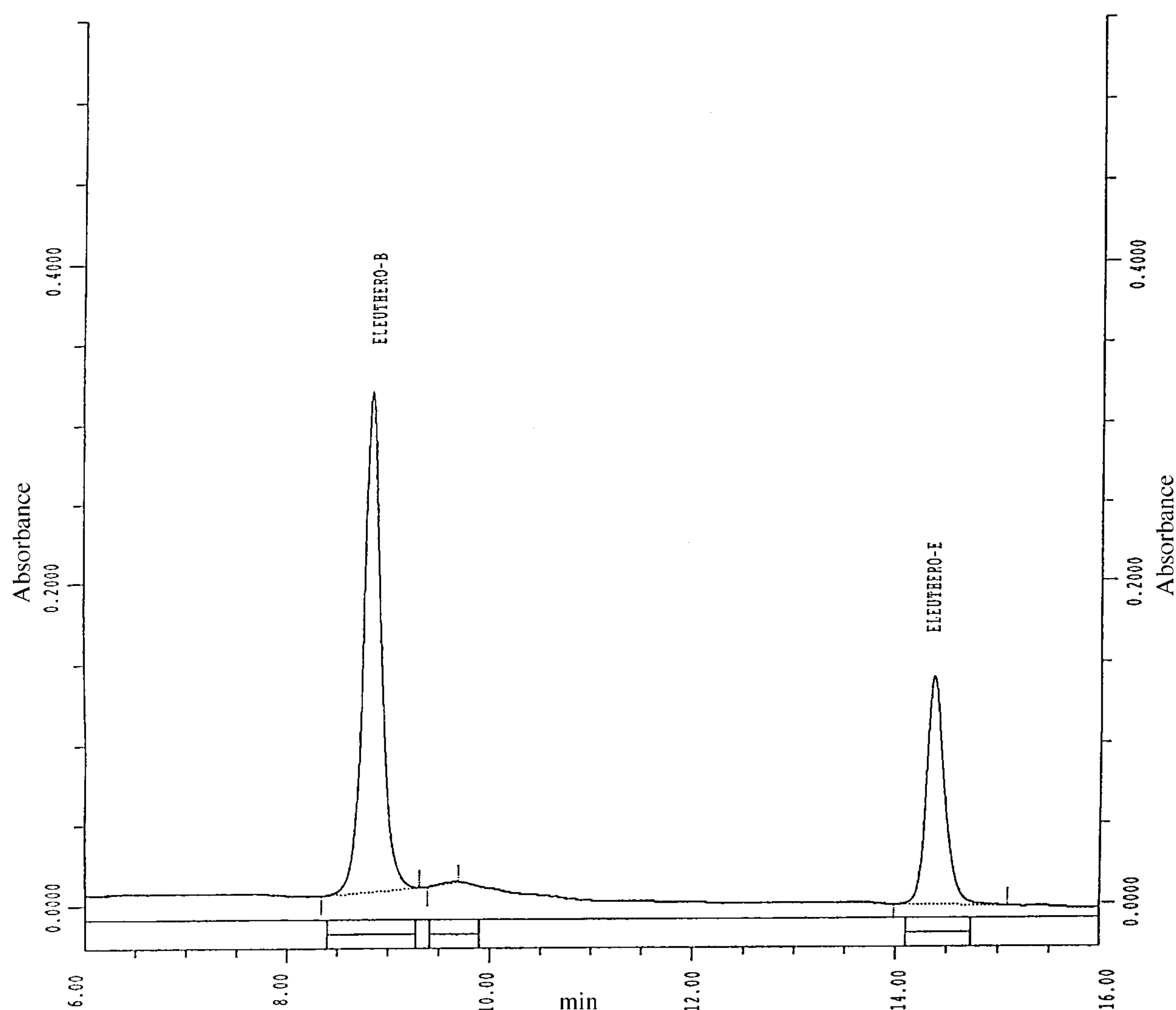

Figure 2. HPLC chromatogram of pure standard eleutherosides B and E (for chromatographic protocol see Experimental section).

where the term "adaptogen" was coined to represent a substance which increases the nonspecific resistance of an organism to adverse influences, while generating a normalizing action on bodily systems (Farnsworth *et al.,* 1985). Such preparations have also been used to alleviate the side effects of anti-cancer drugs (Baranov, 1982).

Eleutherosides, the presumed active constituents of *E. senticosus,* have widely varied structures, and as many as 14 such compounds have been identified and characterized (Awang, 1996), with eleutherosides A–G present at concentrations of 0.6–0.9% in the roots, and 0.6–1.5% in the stems (Farnsworth *et al.,* 1985). Eleutheroside E (Fig. 1; syringaresinol di-*O*-*β*-D-glucoside, identical to liriodendrin, also known as eleutheroside D) is thought to be the pharmacologically most active of these substances (Brekhman and Dardymov, 1969), and constitutes, along with eleutheroside B (Fig. 1; syringin) approximately 80% of the glycosides (Lapchik *et al.,* 1969).

Table 1. Relative standard deviation (RSD) of peak responses for standard Eleutherosides B and E

	Peak Area (AU)	
Analysis number	Eleutheroside B	Eleutheroside E
1	—	24.58
2	226.19	24.82
3	225.93	24.41
4	226.92	24.67
5	230.64	25.00
6	238.55	25.75
MEAN	229.65	24.87
SD	5.33	0.48
%RSD	2.3	1.9

Eleuthero is one of the top 10 selling herbs in the US and recent industry reports have emphasized the importance of standardization of active principles for quality control and consistent pharmacological effect. Fluorimetric (Lapchik *et al.,* 1969), high performance liquid chromatographic (HPLC) (Slacanin *et al.,* 1991), thin layer chromatographic (TLC) (Bladt *et al.,* 1990) and gas chromatography/mass spectrometric (GC/MS) (Awang *et al.,* 1991) methods for the analysis of eleuthero have been reported. The present work describes an improvement in the extraction and quantitative recoveries following HPLC analysis, recognizing the problems with the solubility of eleutheroside E in alcoholic (methanol and ethanol) solvents at ambient temperature. We report an improved extraction procedure reflected in the linearity of the system. The analysis is suitable for a variety of commercial products.

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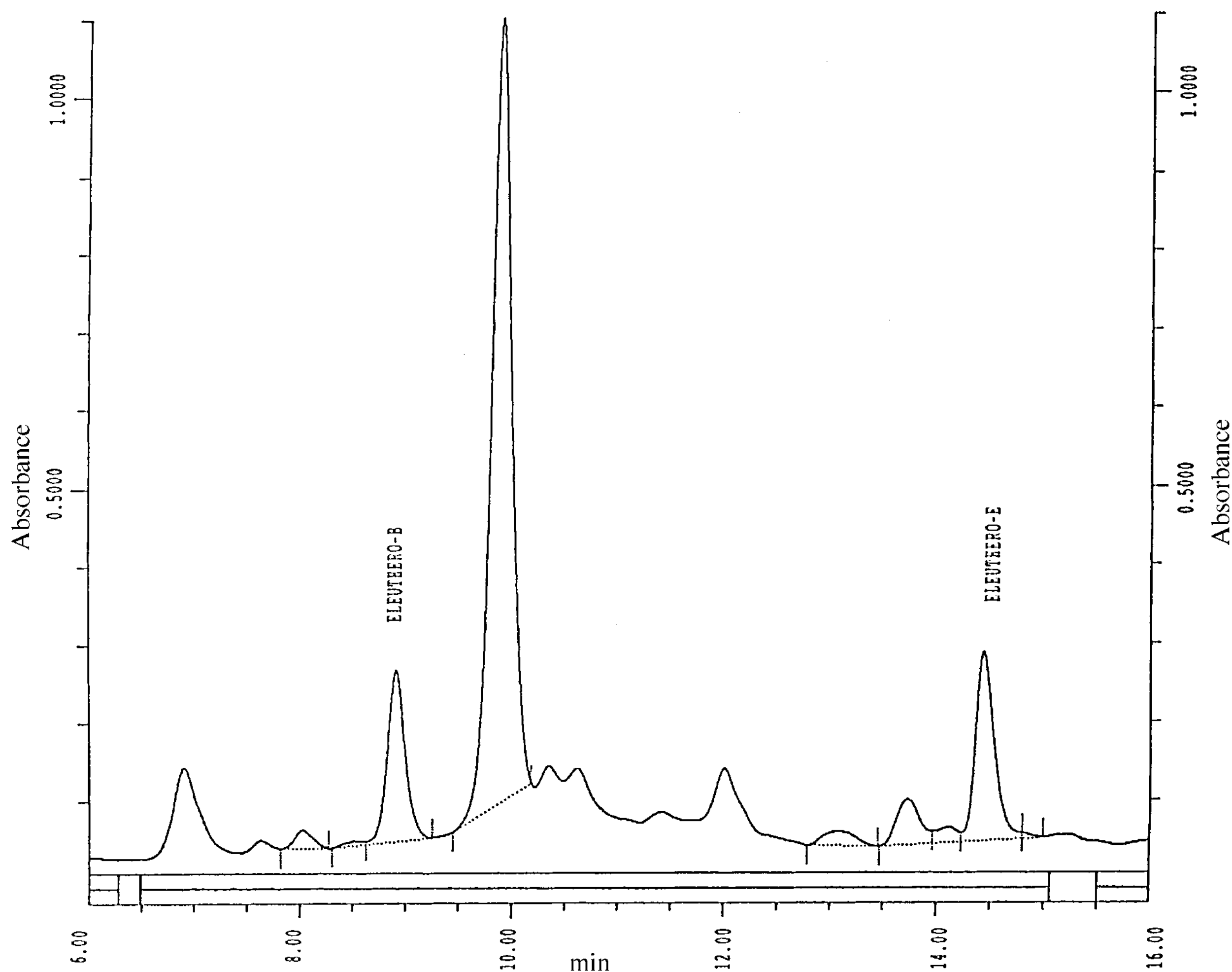

Figure 3. HPLC chromatogram of an extract of authentic eleuthero root showing the presence of eleutherosides B and E (for chromatographic protocol see Experimental section).

EXPERIMENTAL

Reference standards. Eleutheroside B (syringin), eleutheroside B1 (isofraxidin 7-*O*-glucoside) and eleutheroside E (syringaresinol di-*O*-*β*-D-glucoside; liriodendrin) standards were obtained from Professor H. H. S. Fong, University of Illinois at Chicago, USA.

Sample Materials. Authentic roots of *Eleutherococcus senticosus* and commercial samples of *E. senticosus* botanical products were obtained from the American Botanical Council, Austin, TX, USA. Commercial samples were purchased on the North American retail market and submitted to our laboratory only as numbered samples for the determination of eleutheroside content.

Instrumentation. The chromatographic system consisted of a Beckman (Fullerton, CA, USA) System Gold, equipped with a solvent delivery system (module 126), a photodiode array detection system (module 168), and an auto-sampler (module 502) with a 20 µL sample loop. An IBM compatible computer was used for data processing. The instrument was certified by the manufacturers for analytical performances acceptable to the FDA. HPLC analyses were performed with a Beckman Ultrasphere ODS column (250 × 4.6 mm i.d.; 5 µm) fitted with a Beckman ODS precolumn (45 × 4.6 mm i.d.; 5 µm). The

Table 2. Recovery analysis of Eleutheroside B from spiked eleuthero samples

Analysis number	Added Eleutheroside B (mg)	Total Eleutheroside B[a] (mg)	Eleutheroside B recovered (mg)	Recovery (%)
1	0.00	0.11	0.00	—
2	0.50	0.56	0.45	90
3	1.00	0.93	0.82	82
4	2.57	2.53	2.42	94
5	5.00	4.74	4.63	93
6	10.00	8.65	8.54	85

[a] Eleutheroside B in sample was 0.11 mg.

Table 3. Recovery analysis of Eleutheroside E from spiked eleuthero samples.

Analysis number	Added Eleutheroside E (mg)	Total Eleutheroside E[a] (mg)	Eleutheroside E recovered (mg)	Recovery (%)
1	0.00	0.27	0.00	—
2	0.50	0.75	0.48	96
3	1.18	1.26	0.99	84
4	2.50	2.32	2.05	82
5	5.00	3.91	3.64	73
6	10.00	5.83	5.56	56

[a] Eleutheroside E in sample was 0.27 mg.

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Table 4. Analysis of the eleutheroside content of typical commercial eleuthero samples

Sample number/formulation	Sample Amount [g or (mL)]	Eleutheroside content (mg)		Eleutheroside concentration (% w/w or mg/mL)	
		B	E	B	E
1. Capsule	0.440	0.153	0.277	0.03	0.06
2. Capsule	0.600	0.070	0.240	0.01	0.04
3. Capsule	0.995	0.121	1.563	0.01	0.16
4. Powder	0.270	0.142	0.153	0.05	0.06
5. Powder	0.200	0.096	0.426	0.05	0.21
6. Powder	0.800	0.978	0.795	0.12	0.10
7. Powder	0.500	0.061	0.432	0.01	0.09
8. Powder	0.412	1.184	1.021	0.29	0.25
9. Powder	1.050	10.504	6.932	1.00	0.66
10. Powder	0.567	0.209	0.544	0.04	0.10
11. Powder	0.440	1.432	2.179	0.33	0.50
12. Liquid	(2)	0.182	0.296	(0.09)	(0.15)
13. Liquid	(2)	0.252	1.202	(0.13)	(0.60)
14. Liquid	(2)	0.104	0.348	(0.05)	(0.17)
15. Liquid	(2)	0.064	0.020	(0.03)	(0.01)
16. Liquid	(2)	0.258	3.248	(0.13)	(1.62)

mobile phase was made up from solvent A (aqueous 0.05% trifluoroacetic acid in HPLC grade water) and solvent B (acetonitrile). The eleutherosides were separated with a solvent gradient linearly programmed from 10% B to 50% B within 30 min, at a flow-rate of 1 mL/min. Detection was at 220 nm and 0.025 AUFS with a diode array scan from 190 to 400 nm. Linearity was determined by injecting standard solutions of eleutherosides B in the concentration range 1–1000 μg/mL, and of eleutheroside E in the range 4–400 μg/mL. Recovery analyses were carried out in the range 500–10,000 μg, with two determinations performed at each concentration level.

Extraction. Powdered root samples of *E. senticosus* (500 mg) were extracted at 60°C with 20% aqueous methanol (2 × 30 mL), for 30 min each. The combined extract, evaporated to dryness, was dissolved in 20 mL of a mixture of aqueous 0.05% trifluoroacetic acid solution and methanol (1:4) and filtered through a 0.2 μm nylon filter. Liquid samples were evaporated to dryness and treated similarly to the powdered samples. A 20 μL sample of this solution was injected into the chromatographic system and analysed. Co-chromatographic analysis of eleutheroside standards, and the inclusion of their respective response factors (mg/mL/AU) in the chromatographic method, allowed the quantification of the eleutherosides as detected during the course of the analysis.

Recovery. Incremental weights of eleutheroside standards in the range 500–10,000 μg, were added to 500 mg of *E. senticosus* root samples and extracted as for the above sample. Replicate analyses provided total eleutheroside values, which were used to determine recovery rates.

RESULTS AND DISCUSSION

Extraction and analysis of eleutherosides B and E can be highly effective in the assessment of a variety of eleuthero samples for screening purposes, or for the quantification of commercial formulations. Eleutheroside B_1, analysed without further work-up after extraction of eleuthero samples, could not be quantitated, as its peak was masked by that of chlorogenic acid (Slacanin *et al.,* 1991). Attention was focused on the analysis of eleutherosides B and E, which are reported to constitute about 80% of the glycosides in *E. senticosus* (Lapchik *et al.,* 1969). For standard preparations over the range 1–750 μg/mL, eleutheroside B was soluble in methanol: water (80:20); however, eleutheroside E was practically insoluble in this medium. After a variety of tests, it was found that the use of a mixture of aqueous 0.05% trifluoroacetic acid solution and methanol (1:4) was an effective means for the solubilization of both eleutherosides. The chromatogram of standard eleutherosides is shown in Fig. 2 along with that of a typical eleuthero sample in Fig. 3. The linearity of the method was determined by plotting peak area against concentration in the range 1–750 μg/mL for eleutheroside B. The correlation coefficient (r^2) was found to be 0.992. This value was slightly lower (0.985) when the concentration of B was elevated to 1000 μg/mL. The r^2 for eleutheroside E within the range 4–400 μg/mL was 0.992. Method precision gave 2.3% relative standard deviation (RSD) for eleutheroside B and 1.9% RSD for eleutheroside E (Table 1).

Recovery analyses were performed by extracting a uniform amount of eleuthero sample along with added eleutherosides. Recovery of eleutheroside B was generally good and averaged 89% (Table 2). At lower concentrations, eleutheroside E was almost completely extracted at 60°C during the extraction processes (additions of 0.5–2.5 mg of standard to a 0.5g root sample, Table 3), but the progressive drop in percentage recovery as eleutheroside E increased was probably due to approaching the upper limits of the linear range, and to the solubility constraints of eleutheroside E. Following additions of 10 mg to a 0.5 g root sample, the recovery was low (56%).

In examining a selection of commercial samples purchased in the US and Canada (Table 4), it was observed that the eleutheroside E content of all of the samples, except for sample 9, fell in the range of concentration for which good recoveries, based on the

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data in Table 3, were established. Sample 9 appears to be above the historically reported values of eleutheroside content in untreated roots (Bladt *et al.,* 1990), and may represent a fortified preparation. In order to improve recovery in the few samples with very high eleutheroside E content, more solvent should be used, or the sample diluted.

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