SUPPLEMENTARY MATERIAL

Authentication of *Rhodiola rosea*, *Rhodiola quadrifida* and *Rhodiola rosea* liquid extract from the Ukrainian market using HPTLC chromatographic profiles

Kateryna Khokhlova and Oleksandr Zdoryk

*National University of Pharmacy, Kharkiv, Ukraine*

Kateryna Khokhlova, Ph.D., National University of Pharmacy, Department of Technology of Drugs, Pushkinska 53, 61002 Kharkiv, Ukraine. E-mail: kateryna_khokhlova@ukr.net Phone: +38 09 53 55 44 44 Fax: +38 05 77 06 21 84

Kateryna Khokhlova, Ph.D., National University of Pharmacy, Department of Technology of Drugs, Pushkinska 53, 61002 Kharkiv, Ukraine. E-mail: kateryna_khokhlova@ukr.net Phone: +38 09 53 55 44 44 Fax: +38 05 77 06 21 84

Oleksandr Zdoryk, Ph.D., National University of Pharmacy, Department of Quality, Standardization and Certification of Drugs, Pushkinska 53, 61002 Kharkiv, Ukraine. E-mail: oleksandr_zdoryk@ukr.net Phone: +38 09 99 09 46 44 Fax: +38 05 77 06 21 84
Authentication of *Rhodiola rosea*, *Rhodiola quadrifida* and *Rhodiola rosea* liquid extract from the Ukrainian market using HPTLC chromatographic profiles

*Rhodiola rosea* and *Rhodiola quadrifida* are widely distributed and sold in Eastern Europe. The purpose of this paper was to identify *R. rosea*, *R. quadrifida* and *Rhodiola rosea* liquid extract (RRLE) in the Ukrainian market and bring out adulteration cases using chromatographic characterisation by HPTLC. The multiple samples of *R. rosea*, *R. quadrifida* and RRLE were compared; the optimal chromatographic conditions for identification of *R. rosea* and RRLE based on the presence of rosavins and salidroside as well as for identification of *R. quadrifida* based on the presence of salidroside were proposed; the specific HPTLC fingerprints were obtained; the acceptance criteria for each product were set. The adulteration cases for *R. rosea* and RRLE samples were established. The dependence on handling *R. rosea* and presence of rosavins was determined. It was assumed that low-quality raw materials or inefficient technology process were used for RRLE. The consistency of HPTLC fingerprints for *R. quadrifida* samples was established.

Keywords: *Rhodiola rosea*; *Rhodiola quadrifida*; golden root; rosavins; salidroside; authentication; high-performance thin-layer chromatography

**Experimental section**

**Standards**

Salidroside, lot F00110 was purchased from USP (Rockville, USA); rosarin, lot 3328 and rosavin, lot 6358 were purchased from Phytolab (Nuremberg, Germany); gallic acid, lot 021M0035V was purchased from Sigma-Aldrich (Darmstadt, Germany).

**Samples**

11 samples of *R. rosea* (including two dietary supplements in the form of tea) and 10 samples of *R. quadrifida* (including four dietary supplements in the form of tea) were acquired from the market of Ukraine and Russia in 2015. The collected samples of *R. rosea* were dried roots and rhizome of different shapes and sizes: three samples were
whole-piece, seven samples were longitudinally cut, one sample was powdered material. The collected samples of *R. quadrifida* were dried pieces of caudex with dead stems and roots. Three samples of RRLE acquired randomly in the drug stores from different manufacturers of Russia and Ukraine were analyzed. Details of the samples are shown in the supplement material (Table S3).

Before the HPTLC analysis was conducted, all the samples of *R. rosea* and *R. quadrifida* were morphologically authenticated by the specialists of the State Pharmacopoeia of Ukraine and the National University of Pharmacy, Ukraine (Vovk O.G. and Prokopenko Yu.S., PhD). The sample of *R. crenulata*, collected in China, was shared by Dr.E.Reich (China) as a botanical reference material was included in the analysis. The voucher specimens of *R. rosea* (S15144), RRLE (S15129), *R. quadrifida* (S15132) are deposited at the Herbarium of the National University of Pharmacy, Ukraine. Details and voucher specimens numbers are shown in Table S1. The pictures of HRM samples for *R. rosea*, *R. quadrifida*, and *R. crenulata* are shown in the supplement material (Fig. S8).

Preparation of RRLE (laboratory samples used as a reference). These were prepared from the mixture of properly authenticated samples of *R. rosea* S15144, S15145, S15147, S15148, S15149 in the same ratio (1:1) and using the same solvent (Ethanol 40 v/v) as the RRLE samples acquired from manufactures. 100 ml of liquid extract was prepared from 100 g of dry roots and rhizomes of *R. rosea*. Maceration was used as the extraction method (2-day extraction time, room temperature).
**HPTLC Method**

**Methodology**

A comparative study of multiple samples of *R. rosea* and *R. quadrifida*, as well as the commercial preparation of RRLE, was carried out using a HPTLC method. Different chromatographic conditions were compared, the optimal parameters for each product were selected (Table S2), the acceptance criteria were set (Fig.S4-S6). The voucher specimen of potential adulteration such as *R. crenulata* was compared.

**Source of the method**

The HPTLC method for *R. rosea* from USP (USP HMC 2015) was modified; *R. quadrifida* and RRLE were included. The improved HPTLC method from the USP included the modified sample preparation (solvent – ethanol absolute for *R. rosea*, methanol absolute for *R. quadrifida*, ethanol 40% for RRLE), extraction technique – shaking), different combination of markers (rosavin, rosarin, salidroside for *R. rosea* and RRLE; salidroside for *R. quadrifida*) and documentation (additional detection mode at 254 nm before derivatization for *R. rosea* and RRLE and in white light and 254 nm before derivatization, white light after derivatization).

**Chromatographic Conditions**

The sample application, plate layout and developing distance were chosen according to the General Chapter 2.8.25 High-performance thin-layer chromatography (European Pharmacopoeia 9.0). Merck HPTLC silica gel F254 (Darmstadt, Germany). ethyl acetate-methanol-water-formic acid, 77:13:10:2 (v/v/v/v) were used for the analysis. For identification, 3 µL of standard solutions and 5 µL of sample solutions were applied to the plate. Relative humidity was 33%, the chamber was saturated for 20 min, the
temperature was 25°C.

**Visualisation of Fingerprints**

The multiple detection modes used were 254 nm before derivatization and white light after derivatization for *R. rosea* and *R. quadrifida*. The white light before derivatization was additionally used for *R. quadrifida*. The plates were heated at 120°C for 5 min and derivatized by dipping (speed of 3, t=0) while still hot into the aniline-diphenylamine-phosphoric acid reagent. The reagent was prepared as follows: 4.0 g of diphenylamine was dissolved in 160 mL of acetone; then 4 mL of aniline and 30 mL of o-phosphoric acid were carefully added. Other derivatization reagents used in the research were prepared in accordance with the books (Jork et al. 1990, Reich and Schibli 2007).

**Preparation of Samples and Standards**

The sample solution of *R. rosea*: 1.0 g of powdered sample was mixed with 10 mL of ethanol absolute, shaken for 10 min and centrifuged. The supernatant was used as the sample solution.

The sample solution of *R. quadrifida*: 1.0 g of powdered sample was mixed with 10 mL of methanol absolute, shaken for 10 min and centrifuged. The supernatant was used as the sample solution.

The sample solution of RRLE. 9 ml of ethanol 40 v/v were added to 1 ml of RRLE. The mixture was centrifuged and the supernatant was used as the sample solution.

Standard solutions: 1.0 mg/mL of rosavin in methanol; 1.0 mg/mL of rosarin in methanol; 1.0 mg/mL of salidroside in methanol.
**Instruments and Reagents**

**Instruments**

The CAMAG HPTLC system (Muttenz, Switzerland) included a visualizer, an Automatic TLC Sampler 4, an Automatic Developing Chamber 2, and a Scanner 4 controlled by visionCATS software.

**Reagents**

Dichloromethane (for HPLC), lot № 1335297; formic acid (purity 98%), lot № AO343914; ethyl acetate (purity 99.5%), lot № AO355705; acetic acid (purity 99.5%), lot № AO358238; acetone (purity 99%), lot № 1419182; sulfuric acid (purity 96%), lot № AO348254; orthophosphoric acid (85%); sodium hydrogen carbonate, lot № A0206471001 were purchased from Acros Organics (Geel, Belgium). Methanol (HPLC Ultra Gradient Grade), lot № 1042801 was purchased from CarlRoth GmbH (Karlsruhe, Germany); phosphoric acid (purity 85 %), lot № 113196262 was purchased from Roth. Diphenylamine, lot № STBC4473V; aniline, lot № A0348545; antimony (V) chloride, lot № 1413185; 2,6-dibromoquinone-4-chloroimide, lot № BCBJ8822V were purchased from Sigma Aldrich (Darmstadt, Germany). Ethanol absolute (99.8%), lot № 9500090; iron (III) chloride, lot № B951043 145; acetic anhydride, lot № K33146242 were purchased from Merck (Darmstadt, Germany).
| Rhodiola Species | Vernacular names in Russian | Vernacular names in Ukraine | Usage | Distribution |
|------------------|-----------------------------|----------------------------|-------|--------------|
| *Sedum roseum* (L.) Scop. (Syn. *R. rosea* L.) | Rodiola rozovaya (родиола розовая), zolotoy koren (золотой корень – golden root) (Borisova et al. 1939) | Rodiola rozheva (родіола рожева), zolotyi korin (золотий корінь – golden root) (Hrodzinskyi 1990) | It is traditionally used for the temporary relief of symptoms associated with stress, such as fatigue, exhaustion and mild anxiety (Shikov et al. 2014). | In the Russian Federation, the plants of genus Rhodiola are distributed in Altai, Eastern Siberia, Kamchatka, Khabarovsk, Magadan; in Ukraine – the Carpathian Mountains (Borisova et al. 1939, Sokolov 1990, Peshkova et al. 1994). |
| *Sedum quadrifidum* Pall. (Syn. *Rhodiola quadrifida* (Pall.) Fisch. & C.A.Mey.) | Rodiola chetyirehchlenayaya (родиола четырехчленная), rodiola chetyirehmernaya (родиола четырехмерная), Rodiola chetyirehnadreznaya (родиола четырехнадрезная), krasnaya shchetka (красная щетка – red brush*) (Borisova et al. 1939) | Rodiola chotyrokhchelenn (родіола чотирьохчленна) | It is traditionally used as tonic, adaptogen, antidepressant and anti-inflammatory drugs in Asia (Li J et al. 2008) as well as for the treatment of gynaecological diseases in Russia (Sokolov 1990). | *Two other Rhodiola species, such as *R. coccinea* and *R. gelida*, in Russian speaking countries have the similar folk name – red brush for the caudex with dead stems. As the morphological differentiation of *R. quadrifida* and *R. coccinea* is almost impossible, some sources suggest that these are the same species, if chromosomes are not varying (Volkov Igor email to Kateryna Khokhlova, Nov 2, 2016).*
Table S2. Comparison and selection of the appropriate HPTLC chromatographic conditions for *R. rosea*, RRLE and *R. quadrifida*

| Chromatographic conditions | Official monograph for *R. rosea* from USP HMC (2015) | Official monograph for *R. rosea* from USSR Pharmacopoeia (Arzamastsev 1990) | Official monograph for *R. rosea* from SRP (2016) | Additional conditions tested | Proposal for the State Ukraine Pharmacopoeia |
|---------------------------|-------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------|-----------------------------|---------------------------------------------|
| **Stationary phase**      | Silica gel F254 with an average particle size of 5 µm (HPTLC plates) | Silufol UV 254; particle size not specified | Silica gel F254; particle size not specified | – | Silica gel F254 with an average particle size of 5 µm (HPTLC plates) |
| **Mobile phase**          | Ethyl acetate-methanol-water-formic acid (77:13:10:2) (v/v/v/v) | Chloroform-methanol-water (26:14:3) (v/v/v/v) | Chloroform-ethanol absolute-water (25:16:1) (v/v/v) | • Dichloromethane-methanol-water-formic acid (12:4:1:0.5) (v/v/v/v)  
• Dichloromethane-ethanol absolute-water (70:45:6:5) (v/v/v/v)  
• Methanol-acetone (15:2:111:3) (v/v/v)  
• Dichloromethane-methanol (70:9:2) (v/v)  
• Ethyl acetate, acetic acid, formic acid, water (100:11:11:27) (v/v/v/v)  
• Toluene-ethyl acetate-acetic acid (90:10:1) (v/v/v) | Ethyl acetate-methanol-water-formic acid (77:13:10:2) (v/v/v/v) |
| **Test solution**         | 0.1 g/ml in methanol | 0.1 g/ml in methanol | 0.01 g/ml in ethanol 70% | – | 0.1 g/ml in ethanol abs. |
| **Standard solution**     | 1.0 mg/ml of USP rosavin RS in | Any reference standards, just the | 0.4 mg/ml of rosavin in ethanol | rosarin; tyrosol; | 1.0 mg/mL of each reference | 1.0 mg/mL of salidroside |
| **Extraction technique** | Sonication. Usage of supernatant after centrifugation | Heating in a water bath (t=65°) with reverse fridge. Usage of filtrate after filtration | Sonication. Usage of filtrate after filtration | Shaking | Shaking | Usage of supernatant after centrifugation | Usage of supernatant after centrifugation | Shaking |
|-------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------|-----------------|-----------------|-----------------------------------------------|-----------------------------------------------|-----------------|
| **Extraction duration** | 10 min                                              | 20 min                                                                          | 30 min ethanol 70%;                          | 30 min ethanol 70%; | 30 min ethanol 70%; | 10 min ethanol 40 % | 10 min ethanol abs. | 10 min ethanol abs. |
| **Extraction solvent**  | methanol                                            | methanol                                                                        | ethanol 70%;                                  | methanol 60%; ethanol 40%; ethanol abs. | ethanol abs. | ethanol 40 % | ethanol abs. | ethanol abs. |
| **Application volume**  | 3 µl for the rosavin, 5 µl for the test solution and USP **R. rosea** dry extract | 2 µl for the test solution                                                      | 40 µl for the test and standard solutions     | 3 µl for the rosavin, rosarin and salidroside each, 5 µl for the test solution | – | – | – | – |
| **Development**         | -saturated chamber; -development distance – 6 cm;   | -saturated chamber; -development distance – 13 cm                               | -saturated chamber; -development distance – 8-9 cm | –                     | -saturated chamber; -development distance – 6 cm; -relative humidity 33%; -temperature 25° | – | – | – |
| **Derivatization**      | Aniline-diphenylamine-phosphoric acid reagent       | Anisaldehyde-sulfuric acid reagent                                              | -sulfuric acid reagent;                        | Aniline-diphenylamine-phosphoric acid reagent | -fast blue salt B reagent; -2,6-dibromoquinone 4-chloroimide reagent (Gibbs' reagent); -antimony (V) chloride reagent | Aniline-diphenylamine-phosphoric acid reagent | - | - |

- **methanol**: 50 mg/ml of USP **R. rosea** root/rhizome dry extract RS in methanol
- **description of the positions of marker zones due to salidroside (UV 254 nm before derivatization) and due to rosavin (WRT after derivatization)**
- **gallic acid; β-sitosterin**
- **standard (rosavin, rosarin and salidroside)**
- **reference standard (rosavin, rosarin and salidroside)**
- **Extraction technique**: Sonication. Usage of supernatant after centrifugation
- **Heating in a water bath (t=65°) with reverse fridge. Usage of filtrate after filtration**
- **Sonication. Usage of filtrate after filtration**
- **Shaking**
- **Shaking**
- **Usage of supernatant after centrifugation**
- **Usage of supernatant after centrifugation**
- **Extraction solvent**: methanol
- **methanol**
- **ethanol 70%;**
- **ethanol 60%; ethanol 40%; ethanol abs.**
- **ethanol abs.**
- **ethanol 40 %**
- **ethanol abs.**
- **Application volume**: 3 µl for the rosavin, 5 µl for the test solution and USP **R. rosea** dry extract
- **2 µl for the test solution**
- **40 µl for the test and standard solutions**
- **3 µl for the rosavin, rosarin and salidroside each, 5 µl for the test solution**
- **-saturated chamber; -development distance – 6 cm; -relative humidity 33%; -temperature 25°.**
- **-saturated chamber; -development distance – 13 cm**
- **-saturated chamber; -development distance – 8-9 cm**
- **-saturated chamber; -development distance – 6 cm; -relative humidity 33%; -temperature 25°**
- **Aniline-diphenylamine-phosphoric acid reagent**
- **(A) 10% solution of sodium carbonate; (B) diazobenzenesulfonic acid reagent.**
- **Anisaldehyde-sulfuric acid reagent**
- **-sulfuric acid reagent; -fast blue salt B reagent; -2,6-dibromoquinone 4-chloroimide reagent (Gibbs' reagent); -antimony (V) chloride reagent;**
| Examination | WRT after derivatization | -UV-254 nm (before derivatization); -WRT after derivatization | -UV-254 nm (before derivatization); -WRT after derivatization | -WRT before derivatization; -UV-254 nm before derivatization | -UV-254 nm; -WRT after derivatization | -WRT before and after derivatization; -UV-254 nm |
|-------------|--------------------------|-------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|------------------------------------------|---------------------------------------------------|
| Iron (III) chloride (Jork et al. 1990, Reich and Schibli 2007) | | | | | | |
| Sample Number | Sample Description | Details | Supplier, lot | Origin, harvest year |
|---------------|--------------------|---------|---------------|---------------------|
| S15141        | *Rhodiola rosea*   | Dried rhizome/root, whole-piece form | ‘Agrofirm’, Ukraine, Lot 0114 | Altaj, Russia, 2014 |
| S15142        | *Rhodiola rosea*   | Dried rhizome/root, chopped form, herbal tea, dietary suppl. | ‘Belowodje’, Russia, Lot 101014 | Russia, 2014 |
| S15143        | *Rhodiola rosea*   | Dried rhizome/root, chopped form, herbal tea, dietary suppl. | ‘Strength of Nature’ Penza, Russia, Lot 15102014 | Altaj, Russia, 2014 |
| S15144        | *Rhodiola rosea*   | Dried rhizome/root, chopped form | ‘Agrofirm’, Ukraine, Lot 0214 | Altaj, Russia, 2014 |
| S15145        | *Rhodiola rosea*   | Dried rhizome/root, chopped form | Retailer, Ukraine, not specified | Altaj, Russia, 2014 |
| S15146        | *Rhodiola rosea*   | Dried rhizome/root, powdered form | ‘Aim’, Ukraine, not specified | Not specified, 2014 |
| S15147        | *Rhodiola rosea*   | Dried rhizome/root, chopped form | ‘World of, plants’, Ukraine, Lot 114 | Altaj, Russia, 2014 |
| S15148        | *Rhodiola rosea*   | Dried rhizome/root, chopped form | Retailer, Ukraine, Lot 012014 | Altaj, Russia, 2014 |
| S15149        | *Rhodiola rosea*   | Dried rhizome/root, chopped form | Retailer, Ukraine, Lot 022014 | Altaj, Russia, 2014 |
| S15150        | *Rhodiola rosea*   | Dried rhizome/root, whole-piece form | ‘World of plants’, Ukraine, Lot 214 | The Carpathian, Ukraine, 2014 |
| S15151        | *Rhodiola rosea*   | Dried rhizome/root, whole-piece form | ‘Aim’, Ukraine, not specified | Not specified, 2013 |
| S15152        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots | ‘World of plants’, Ukraine, Lot 314 | Altaj, Russia, 2014 |
| S15153        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots | Retailer, Ukraine, Lot1 | Altaj, Russia, 2014 |
| S15154        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots | Retailer, Ukraine, Lot2 | Altaj, Russia, 2014 |
| S15155        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots | ‘Drugstore of herbals’, Lot 012014 | Altaj, Russia, 2014 |
| S15156        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots | Retailer, Ukraine, not specified | The Carpathian, Ukraine, 2014 |
| S15157        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots, herbal tea | ‘PhytoBioTechnology’, Kiev, Ukraine, Lot 012 | Not specified, 2014 |
| S15158        | *Rhodiola quadrifida* | Dried pieces of caudex/dead stems/roots | ‘Agrofirm’, Ukraine | Altaj, Russia, 2014 |
| S15159        | *Rhodiola*         | Dried pieces of | ‘Herbs of Altaj’, | Altaj, Russia, |
| Code   | Species           | Description                                      | Lot    | Country/Region  |
|--------|-------------------|--------------------------------------------------|--------|-----------------|
| S15131 | *Rhodiola quadrifida* | Dried caudex/dead stems/roots, powdered form, herbal tea | Lot 102013 | Altaj, Russia, 2014 |
| S15132 | *Rhodiola quadrifida* | Dried caudex/dead stems/roots, powdered form, herbal tea | ‘Herbs of Altaj’, Altaj, Russia, Lot 102013 | Altaj, Russia, 2013 |
| S15127 | *Rhodiola rosea* liquid extract | Liquid extract, ratio – 1:1, solvent – ethanol 40 v/v | ‘Camelia’, Moscow, Russia, Lot 010215, 0320 | Russia, not specified |
| S15128 | *Rhodiola rosea* liquid extract | Liquid extract, ratio – 1:1, solvent – ethanol 40 v/v | ‘Vifiteh’, Moscow, Russia, Lot 010115, 0220 | Russia, not specified |
| S15129 | *Rhodiola rosea* liquid extract | Liquid extract, ratio – 1:1, solvent – ethanol 40 v/v | ‘Biolik’, Ladigin, Ukraine, Lot 030611 | Not specified |
| S15167 | *Rhodiola rosea* liquid extract, laboratory sample | Prepared from a mixture of *R. rosea* samples: S15144, S15145, S15147, S15148, S15149, extraction method maceration | - | - |
| S15184 | *Rhodiola crenulata* | Dried rhizome/root | Shared by Dr.E.Reich | China, not specified |
Figure S1. Selection of the marker substances and characteristic HPTLC fingerprinting for *Rhodiola* species. Tracks: 1 – rosavin; 2 – rosalin; 3 – salidroside; 4 – gallic acid; 5 – tyrosol; 6 – *R. rosea*, 7 – *R.crenulata*, 8 – *R.quadrifida*.

Detection a: 254 nm, before derivatization; detection b: white light, after derivatization.
Figure S2. Selection of the mobile phase and detection mode

A. MP: Chloroform, methanol, water (26:14:3), Derivatization: aniline-diphenylamine-phosphoric acid reagent; Tracks: 1– rosavin, rosarin, salidroside (Rf ↑); 2 – *R. rosea*, ethanol abs.extract; 3 – *R. rosea*, methanol extract; 4 – *R. crenulata*.

B. MP: Ethyl acetate, methanol, water, formic acid (77:13:10:2); Derivatization: aniline-diphenylamine-phosphoric acid reagent; Tracks: 1 – rosavin, rosarin, salidroside (Rf ↑); 2 – *R. rosea*; 3 – *R. crenulata*.

C. MP: Dichloromethane, ethanol absolute, water (70:45:6.5); Derivatization: aniline-diphenylamine-phosphoric acid reagent; Tracks: 1 – rosavin, rosarin, salidroside (Rf ↑); 2 – *R. rosea*; 3 – *R. crenulata*; 4 – *R. quadrifida*.

D. MP: Ethyl acetate, acetic acid, formic acid, water (100:11:11:27); Derivatization: aniline-diphenylamine-phosphoric acid reagent; Tracks: 1– rosavin, rosarin, salidroside (Rf ↑); 2 – *R. rosea*; 3 – *R. crenulata*; 4 – *R. quadrifida*.

E. MP: Toluene, ethyl acetate, acetic acid (90:10:1); Derivatization: Anisaldehyde-sulfuric acid reagent; Tracks: 1– rosavin, rosarin, salidroside (Rf ↑); 2 – *R. rosea*; 3 – *R. crenulata*.

F. MP: Dichloromethane, methanol, water, formic acid (12:4:1:0.5); Derivatization: aniline-diphenylamine-phosphoric acid reagent; Tracks: 1– rosavin, rosarin, salidroside (Rf ↑); 2 – *R. rosea*; 3 – *R. crenulata*; 4 – *R. quadrifida*.

Detection a: 254 nm, before derivatization; detection b: white light, after derivatization; detection c: 366 nm, after derivatization.
Tracks: 1 – rosavin, rosarin, salidroside (Rf ↑); 2-6 – samples of \textit{R.rosea} (solvents were: 2 – methanol 60%, 3 – methanol abs., 4 – ethanol abs., 5 – ethanol 70%; 6 – ethanol 40%); 7-9 – samples of \textit{R.crenulata} (solvents were: 7 – methanol 60%, 8 – methanol abs., 9 – ethanol abs.); 10-12 – samples of \textit{R.quadrifida} (solvents were: 10 – methanol 60%, 11 – methanol abs., 12 – ethanol abs.).

Detection a: 254 nm, before derivatization; detection b: white light, after derivatization.
Figure S4. Table description and the typical chromatogram of *R. rosea*.

Tracks: 1 – rosvain, rosarin, salidroside (Rf ↑); 2-8 – different samples of *R. rosea*.

Detection a: 254 nm, before derivatization; detection b: white light, after derivatization.
Figure S5. Table description and the typical chromatogram of RRLE
Tracks: 1 – rosavin, rosarin, salidroside (Rf ↑); 2-5 – different samples of RRLE.

Detection a: 254 nm, before derivatization; detection b: white light, after derivatization.
Figure S6. Table description and the typical chromatogram of *R. quadrifida*

Tracks: 1 – rosavin, rosarin, salidroside (Rf ↑); 2-11 – different samples of *R. quadrifida.*

Detection a: white light, before derivatization; detection b: 254 nm, before derivatization; detection c: white light, after derivatization.
Figure S7. Discrimination of falsification products of *R. rosea* and RRLE. MP: ethyl acetate, methanol, water, formic acid (77:13:10:2).

Tracks: 1 – rosavin, rosarin, salidroside (Rf ↑); 2-12 – samples of *R. rosea* roots and rhizomes (among them 3-6, 8-10 – cut HRM; 2, 11, 12 – whole-piece, 7 – powder); 13-15 – samples of RRLE from different manufacturers; 16-18 – samples of RRLE prepared in laboratory conditions from authenticated HRM.

Detection a: 254 nm, before derivatization; detection b: white light, after derivatization.
Figure S8. Pictures of HRM of different Rhodiola species R. rosea, cut material (A); R. rosea, whole-piece sample (B); R. crenulata, cut material (C); R. quadrifida, the piece of samples (D).
References

Arzamastsev AP, Kosyireva NS, editors. 1990. Gosudarstvennaya farmakopeya SSSR [State SSSR Pharmacopoeia]. 11th ed. Moskva: Meditsina. Russian

Borisova AG, Komarov VL, Krishtofovich AN, Lozina-Lozinskaya AS, Maleev VP. 1939. Flora SSSR [Flora SSSR]. IX ed. Moskva: Izdatelstvo akademii nauk SSSR. Russian.

European Pharmacopoeia. 2016. 9th ed. Strasbourg: Council of Europe; p. 295.

Hrodzinskyi 1990 Hrodzinskyi, editor. 1990. Likarstvo rosliny: entsyklopedychnyi dovidnyk [Herbal plants: encyclopedic reference book]. Kïïv: Holovna redaktsiia ukrainskoï radianskoï entsyklopedii; p. 377–378. Ukrainian

Jork H, Funk W, Fischer W, Wimmer H. 1990. Physical and Chemical Detection Methods: Fundamentals, Reagents, in: Thin-Layer Chromatography Reagents and Detection Methods. Weincheim: VCH.

Li J, Yi T, Lai HS, Xue D, Jiang H, Peng HC, Zhang H. 2008. Application of microscopy in authentication of traditional Tibetan medicinal plants five Rhodiola (Crassulaceae) alpine species by comparative anatomy and micromorphology. Microsc Res Tech. 71:11–19.

Peshkova GA, Malıyishev LI, Nikiforova OD, Doronkin VM, Ovchinnikov SV. 1994. Flora Sibiri. Berberidaceae-Grossulariaceae. [Flora of Siberia. Berberidaceae-Grossulariaceae]. 7th ed. Novosibirsk: Nauka. Russian.

Reich E, Schibli A. 2007. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Stuttgart: Thieme.

Shikov AN, Pozharitskaya ON, Makarov VG, Wagner H, Verpoorte R, Heinrich M. 2014. Medicinal Plants of the Russian Pharmacopoeia; Their history and applications. J. Ethnopharmacol. 481-536.

Sokolov PD, editor. 1990. Rastitelnyie resursyi SSSR. Tsvetkovyie rasteniya, ih himicheskiy sostav, ispolzovanie: Caprifoliaceae-Plantaginaceae. [Flowering plants, its chemical composition, application: Caprifoliaceae-Plantaginaceae]. 5th ed. Leningrad: Nauka. Russian.

State Russian Pharmacopoeia. XIII ed. [SRP]. 2016. Moscow: NZSMP; [accessed 10.10.18]. http://pharmacopoeia.ru/fs-2-5-0036-15-rodioly-rosovaj-kornevishha-i-
USP Herbal Medicines Compendium [USP HMC]. 2015. Rockville (MD): USP;
[accessed 10.7.15]. http://hmc.usp.org/