High-Performance Thin-Layer Chromatography: A Fast and Efficient Fingerprint Analysis Method for Medicinal Plants

HPTLC Fingerprint of Ginsenosides

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Ginsenosides are triterpene saponins. Most ginsenosides are composed of a dammarane skeleton (17 carbons in a four-ring structure) with various sugar moieties (*e.g.* glucose, rhamnose, xylose and arabinose) attached to the C-3 and C-20 positions.

Over 30 ginsenosides have been identified and classified into two categories:

- 20(S)- protopanaxadiol (PPD) (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, Rs1)
- 20(S)-protopanaxatriol (PPT) (Re, Rf, Rg1, Rg2, Rh1). The difference between PPTs and PPDs is the presence of a carboxyl group at the C-6 position in PPDs.

Moreover, several rare ginsenosides, such as the ocotillol saponin F11 (24-R-pseudoginsenoside) and the pentacyclic oleanane saponin Ro (3,28-o-bisdesmoside) have also been identified. Asian ginseng (*Panax ginseng*) is commonly known as the true ginseng.

In this article, HPTLC methods suitable for the analysis of ginsenosides are presented, using CAMAG equipment, Merck TLC plates, analytical standards and extract reference materials. The extract reference materials are manufactured by HWI Analytik and exclusively distributed by Merck Sigma-Aldrich.

Detection of ginsenosides in the HPTLC fingerprint of different Panax species (roots and root extracts) is obtained by following the HPTLC methods of the Ph. Eur. monograph,[1] USP DSC 2015 monograph[2] and the method of the HPTLC Association (International Association for the Advancement of HPTLC)[3] by comparison of the R_F values and colors of reference substances and matching zones in the root extract. Depending on the regulation followed, one of the three methods of identification can be used.

Recommended CAMAG Devices:

Automatic TLC Sampler (ATS 4), Automatic Developing Chamber (ADC 2), TLC Visualizer 2, Chromatogram Immersion Device 3, TLC Plate Heater 3, and visionCATS

Derivatization Reagent:

Anisaldehyde[1] or sulfuric acid reagent[2,3]

Sample:

0.015 g/mL extract (HWI extract) in 70% methanol[1]

Note: Deviation from methods 2 and 3 for the sample preparation

Standards:

Standard solutions of ginsenosides were prepared in a concentration of 0.5 mg/mL in methanol.

Note: Deviation from method 3 for the application volumes

Chromatography Following USP <203> [4]:

Stationary phase: HPTLC Si 60 F_{254} 20 x 10 cm (Merck)

Sample application: 4 µL each of test solution and 2 µL of standards are applied as 8 mm bands, 8 mm from lower edge, 20 mm from the left edge[1, 3]

Note: Deviation from method 3 for the sample preparation and the application volume of reference and test solutions

4 µL each of test solution and 4 µL of standards are applied as 8 mm bands, 8 mm from lower edge, 20 mm from the left edge[2]

Note: Deviation from method 2 for the sample preparation

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Developing solvent: Ethyl acetate – water – butanol 25:50:100 (*v/v/v*) - upper layer[1]

Dichloromethane – ethanol – water 70:45:6.5 (*v/v/v*)[2]

Chloroform – ethyl acetate – methanol – water 15:40:22:9 (*v/v/v/v*)[3]

Development: In the ADC 2 with an unsaturated chamber and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride[1]

Development is performed with ADC 2, saturated for 20 minutes with the developing solvent (filter paper). Prior to the development the plate is conditioned for 10 min to a relative humidity of 33% (with a saturated solution of $MgCl_2$).[2, 3]

Developing distance: 70 mm (from lower edge)[1, 2]
80 mm (from lower edge)[3]

Plate drying: 5 min in a stream of cold air

Derivatization: The plate is immersed (immersion speed: 3 cm/s, immersion time: 0 s) into anisaldehyde reagent (mixture of 0.5 mL of *p*-anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of sulfuric acid) with the Chromatogram Immersion Device 3 and heated for 5 min at 105°C.[1]

The plate is immersed (immersion speed: 3 cm/s, immersion time: 0 s) into sulfuric acid reagent (10% in methanol) with the Chromatogram Immersion Device **3** and heated for 5 min at 100 °C.[2, 3]

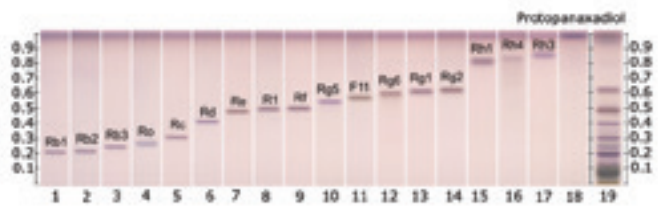
Evaluation: Documentation under method 3 is with 100 °C white light[1, 2, 3] and UV 366 nm[2, 3] after derivatization with the TLC Visualizer 2

Results and Discussion:

HPTLC chromatograms of ginsenoside standards and a *Panax ginseng* root extract

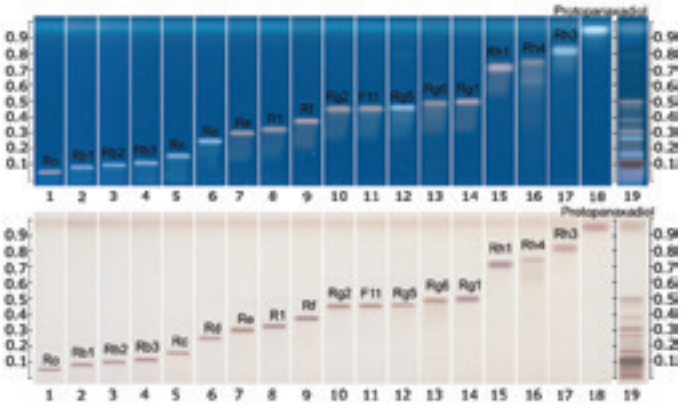
HPTLC chromatograms after derivatization. Tracks 1-17: ginsenosides, track 18: protopanaxadiol, track 19: *Panax ginseng* root extract (article no.: 05115001 batch: HWI01294)

Method 1: According to the Ph. Eur.[1]



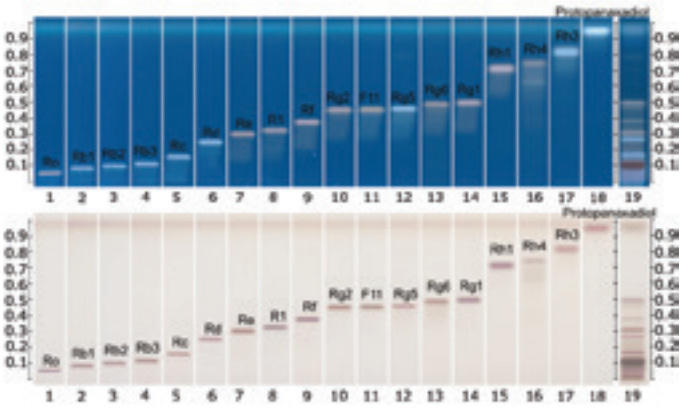
HPTLC chromatograms under white light after derivatization

Method 2: According to the Tienshi ginseng method from[2]



HPTLC chromatograms under UV 366 nm and under white light after derivatization

Method 3: According to the HPTLC Association[3]

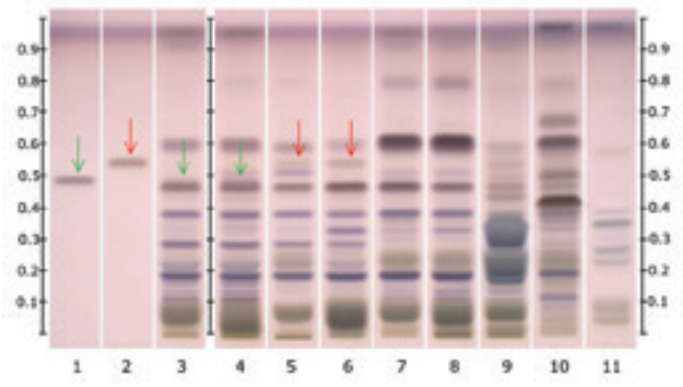


HPTLC chromatograms under UV 366 nm and under white light after derivatization.

HPTLC chromatograms of plants containing ginsenosides (different Panax species)

P. ginseng, P. quinquefolium, P. notoginseng, P. japonicus, P. vietnamensis roots and root extracts were collected and analyzed. The *P. ginseng* root extract (article no.: 0511-50-01 batch: HWI01294) was used as botanical reference material to identify Asian ginseng (the ginsenoside Rf should be present and F11 absent).

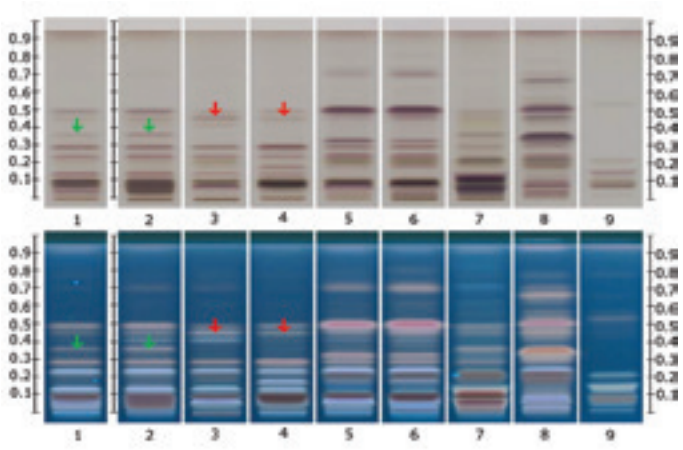
Method 1: According to the Ph. Eur.[1]



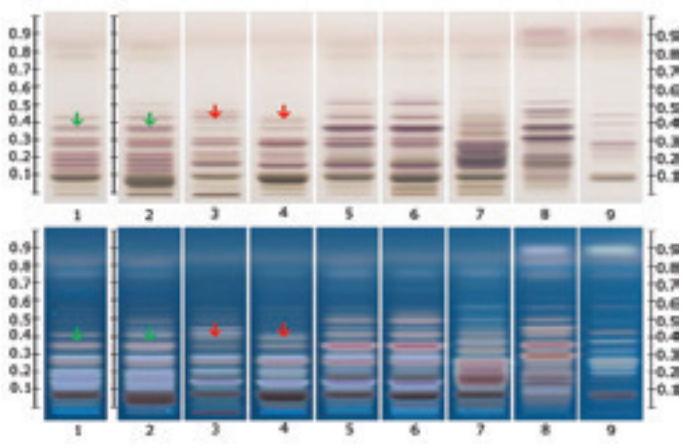
HPTLC chromatogram under white light after derivatization.

Track 1: ginsenoside Rf (green arrow); **2:** ginsenoside F11 (red arrow); **3:** *P. ginseng* root extract (article no.: 0511-50-01 batch: HWI01294, ginsenoside Rf highlighted with the green arrow); **4:** P. ginseng root (ginsenoside Rf highlighted with the green arrow); **5:** *P. quinquefolium* root extract (American ginseng, ginsenoside F11 highlighted with the red arrow); **6:** *P quinquefolium* root (American ginseng, ginsenoside F11 highlighted with the red arrow); **7:** *P. notoginseng* root extract; **8:** *P. notoginseng* root; **9:** *P. japonicas* root; **10:** *P. vietnamensis* root; **11:** wild Vietnamese ginseng root.

Method 2: According to the Tienshi ginseng method from[2]



Method 3: According to the HPTLC Association[3]



Method 2 & 3 Parameter:

HPTLC chromatograms under white light and UV 366 nm after derivatization with ginsenoside Rf (green arrows) and ginsenoside F11 (red arrows).

Track 1: *P. ginseng* root extract (article no.: 05115001 batch: HWI01294, ginsenoside Rf highlighted with the green arrows); **2:** *P. ginseng* root (ginsenoside Rf highlighted with the green arrows); **3:** *P. quinquefolium* root extract (American ginseng, ginsenoside F11 highlighted with the red arrows); **4:** *P quinquefolium* root (American ginseng, ginsenoside F11 highlighted with the red arrows); **5:** *P. notoginseng* root extract; **6:** *P. notoginseng* root; **7:** *P. japonicas* root; **8:** *P. vietnamensis* root; **9:** wild Vietnamese ginseng root.

All shown methodologies are suitable for detection of ginsenosides in different Panax species. Ginsenoside Rf is unique to Asian ginseng while F11 is found exclusively in American ginseng. Thus the Rf/F11 ratio is used as a phytochemical marker to distinguish American ginseng from Asian ginseng. In the botanical reference material used (*Panax ginseng* root extract, article no.: 0511-50-01) the presence of Rf and absence of F11 could be confirmed with all methods and it is therefore suited for identification of Asian ginseng.

Methods 1 and 2 have the advantage in that they are in accordance with official monographs in the pharmacopoeias (Ph. Eur. resp. USP). Method 3 is an alternative method provided by the HPTLC Association. The method is improved for the separation of Rf and F11 to better distinguish American and Asian ginseng. The derivatization with sulfuric acid reagent (methods 2 and 3) leads to different colored zones under UV 366 nm, useful for identification.

Featured Products

Description	Package Size	Cat. No.
Analytical Standards		
Ginsenoside Rb1*	10mg	**170580**
Ginsenoside Rb2	10mg	**41868**
Ginsenoside Rb3	10mg	**42635**
Ginsenoside Rc	5mg	**44987**
Ginsenoside Rd	10mg	**01518**
Ginsenoside Re*	10mg	**03000590**
Ginsenoside Re	10mg	**77960**
Ginsenoside Rf*	10mg	**01580590**
Ginsenoside Rg1*	10mg	**00370580**
Ginsenoside Rg2	10mg	**08171**
Ginsenoside Rg3	10mg	**64139**
Ginsenoside Rg5	5mg	**43016**
Ginsenoside Rh1	10mg	**56805**
Ginsenoside Rh3	5mg	**43084**
Ginsenoside Rh4	5mg	**42776**
Ginsenoside Ro	10mg	**94381**
Notoginsenoside R1	10mg	**77089**
Protopanaxadiol	10mg	**62685**
Protopanaxatriol	10mg	**42476**
Pseudoginsenoside F11	10mg	**67530**

*HWI reference standard

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Featured Products (cont.)

Description	Package Size	Cat. No.
Extract Reference Material		
Panax ginseng extract Quantitative Markers: Ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg2 Qualitative Markers**: Ginsenoside Rb1 and Rg1	500mg	**05115001**
TLC Plates		
HPTLC glass plate Silica gel 60 F254 20×10 cm	50 Plates	**1.05642**

(**: traceable to HWI primary pharmaceutical standards)

References

1. Ginseng root: Monograph in Ph. Eur. 8.0, 01/2008:2383. European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France.
2. Tienchi Ginseng Root and Rhizome Dry Extract: Monograph in USP 40-NF35 (2017). United States Pharmacopeial Convention, Rockville, MD, USA.
3. HPTLC identification method for *Panax ginseng*, HPTLC Association (www.hptlc-association.org) (accessed May 22, 2016)
4. <203> High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin in USP 39-NF34. (2016), United States Pharmacopeial Convention, Rockville, MD, USA

Find all available analytical standards for phytochemicals listed on **SigmaAldrich.com/medicinalplants**

For all ginsenoside standards see **SigmaAldrich.com/panax**

An overview of all plant extract reference materials can be found at **SigmaAldrich.com/plantextracts**

NUTRITIONAL SUPPLEMENTS

New Phytochemical Standards from HWI Pharma Solutions

Matthias Nold, Product Manager, Reference Materials, **matthias.nold@sial.com**

We are proud to be an exclusive distributor of the reference standards manufactured by HWI-Pharma Solutions in Rülzheim (Germany).[1] Since the launch of the first series of products in January 2011, the product range has been continuously expanded and currently consists of more than 120 products.

The quantitative value is determined by **quantitative NMR** (qNMR).[2] This is a direct relative method, which is increasingly used for the quantification of organic compounds, as an alternative to the much more laborious mass balance approach. The certificate delivered with these products also contains a chromatographic purity value.

Recently, several new products have been added to the portfolio (see **Table 1**). A complete listing can be found online at **SigmaAldrich.com/phytopharma**.

Table 1.Newly added HWI reference standards of phytopharmaceuticals

Description	Package Size	Cat. No.
Benzyl acetate	100mg	**05880595**
Berberine chloride	50mg	**00900585**
Carminic acid	25mg	**03320585**
(±)-β-Citronellol	100mg	**05630590**
Ectoine	100mg	**02380595**
Hydroxyectoine	100mg	**02390595**
Isoxanthohumol	25mg	**05890580**
DL-Kavain	25mg	**05790585**
Alpha-onocerin	10mg	**05800590**
Patchouli alcohol	10mg	**05690595**

References:

1. G. Förster, F. Michel, M. Nold; Analytix 1/2010 page 11.
2. M. Veith; Analytix 1/2010 page 14.