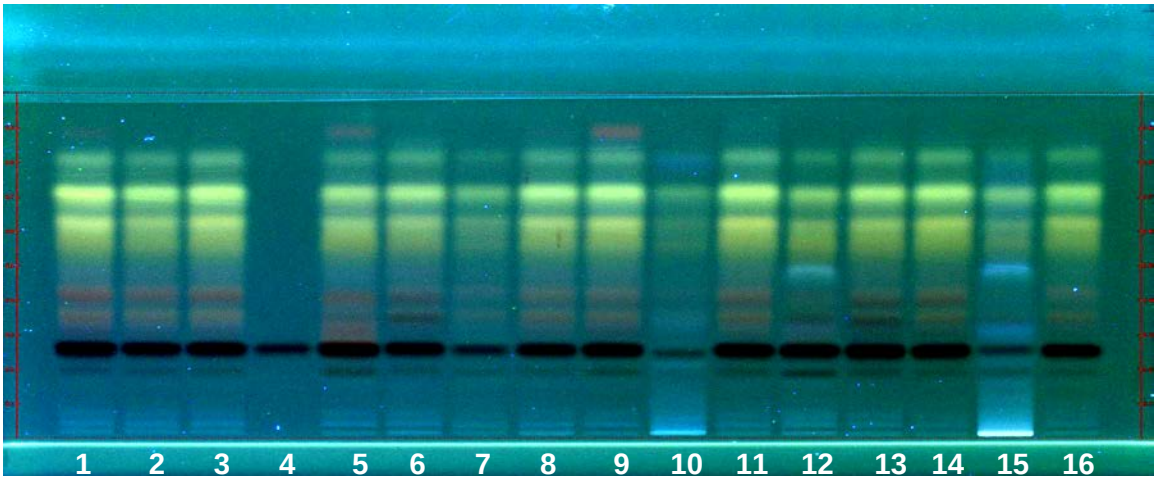
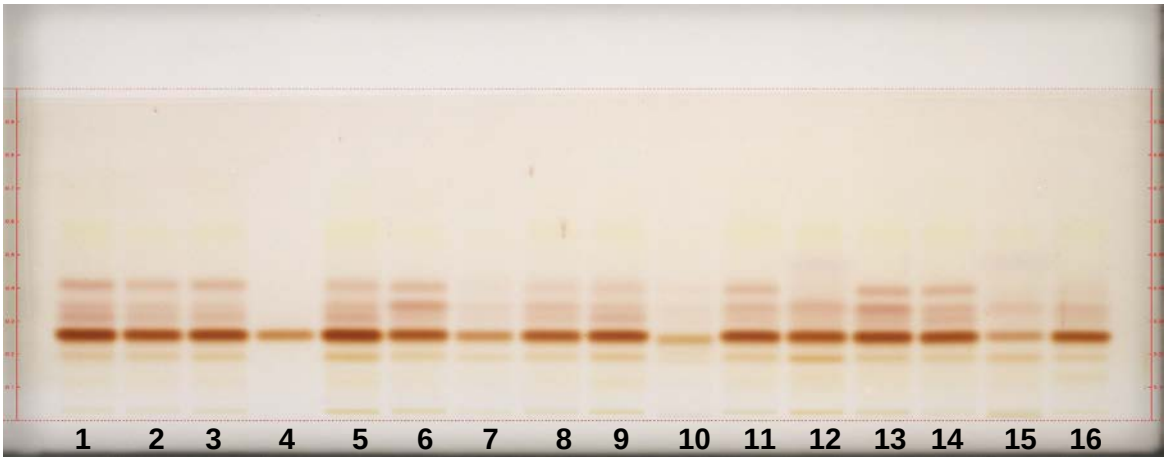
Trigonella foenum-graecum Seed — Identification

Thin-Layer Chromatography – Amino Acids Profile



A



B

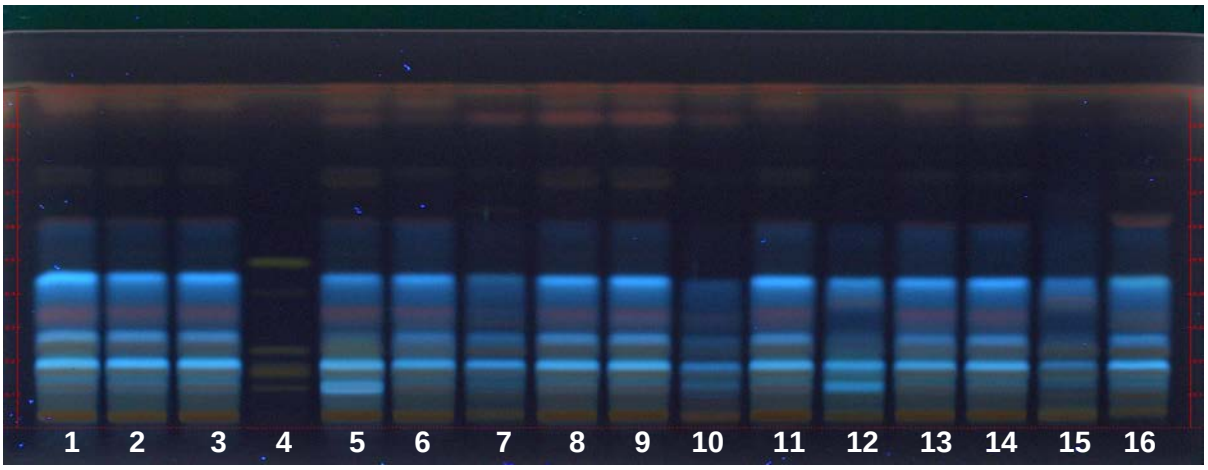
Typical HPTLC Chromatograms

These chromatograms are supplied for information only

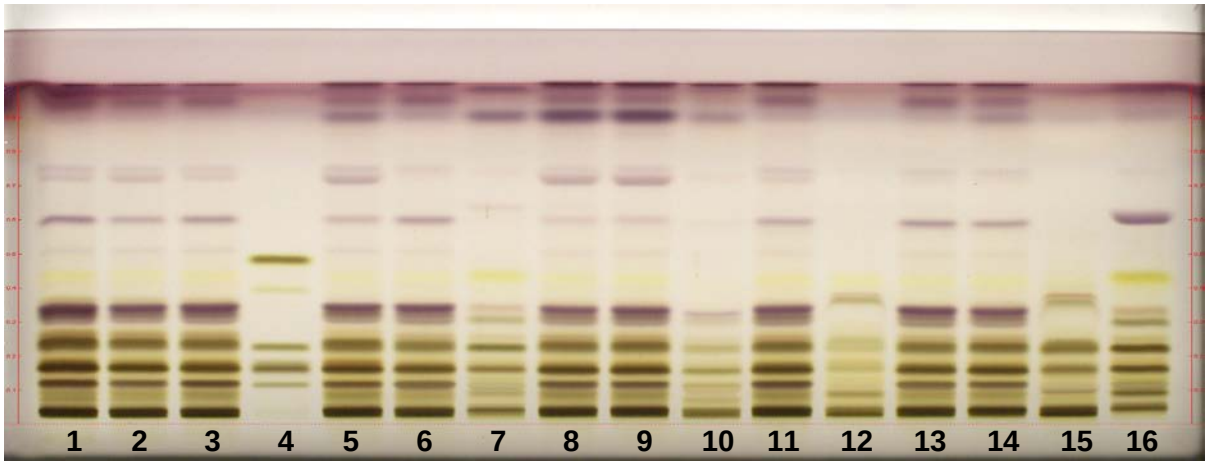
Track assignment: 1-3) *Trigonella foenum-graecum* Seed, commercial samples; 4) USP 4-Hydroxyisoluecine RS (0.5 mg/mL); 5-13) finished products (capsules and tablets); 14-16) liquid extracts

Sample solutions:	according to the monograph
Standard solutions:	in methanol
Plate:	HPTLC, Si 60 F_{254}
Saturation time:	20 minutes
Application volume:	2 µL, as 8-mm bands
Relative Humidity:	about 33%
Temperature:	25°
Developing solvent system:	*n*-Butanol, acetic acid, and water (7:2:1)
Developing distance:	6 cm
Derivatization reagent:	ninhydrin reagent – 0.3 g of ninhydrin, 95 mL of isopropanol, and 5 mL of glacial acetic acid
Detection:	derivatize, heat at 100-105º for 2 min, and examine under (A) UV light at 366 nm and (B) visible light.

Thin-Layer Chromatography – Steroidal Saponins Profile



A



B

Typical HPTLC Chromatograms

These chromatograms are supplied for information only

Track assignment: 1-3) *Trigonella foenum-graecum* Seed, commercial samples;

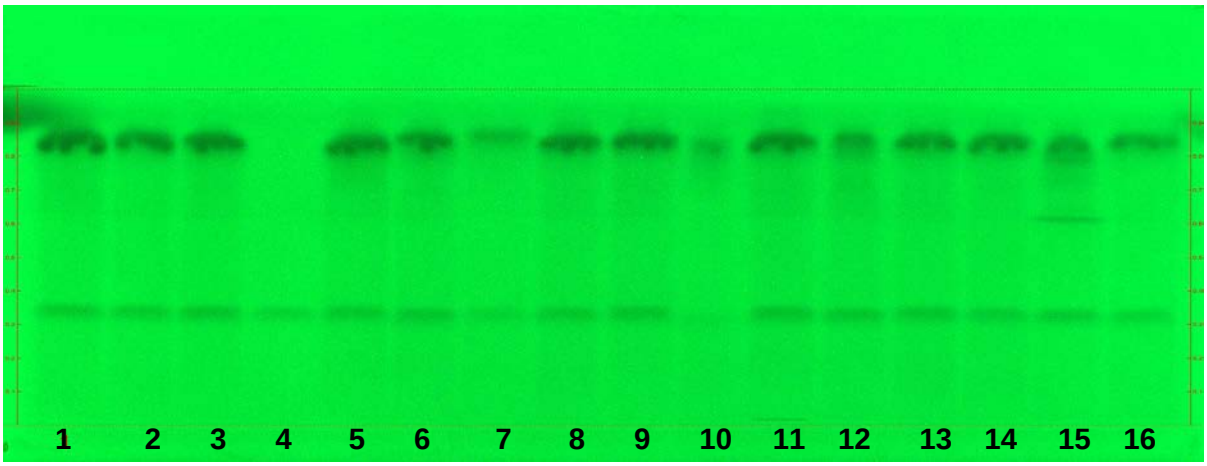
4) fructose, protogracillin, protodioscin and dioscin (two bands), 0.5 mg each/mL (with increasing R_F);

5-13) finished products (capsules and tablets); 14-16) liquid extracts.

Sample solutions:	according to the monograph
Standard solutions:	in methanol
Plate:	HPTLC, Si 60 F_{254}
Saturation time:	20 minutes
Application volume:	2 µL, as 8-mm bands
Relative Humidity:	about 33%
Temperature:	25°
Developing solvent system:	chloroform, methanol and water (18:8:1)
Developing distance:	6 cm
Derivatization reagent:	anisaldehyde reagent – 85 mL of ice-cooled methanol mixed with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of *p*-anisaldehyde

Detection:	derivatize, heat at 100º for 2-3 min, and examine under (A) UV light at 366 nm and (B) visible light

Thin-Layer Chromatography – Presence of Trigonelline



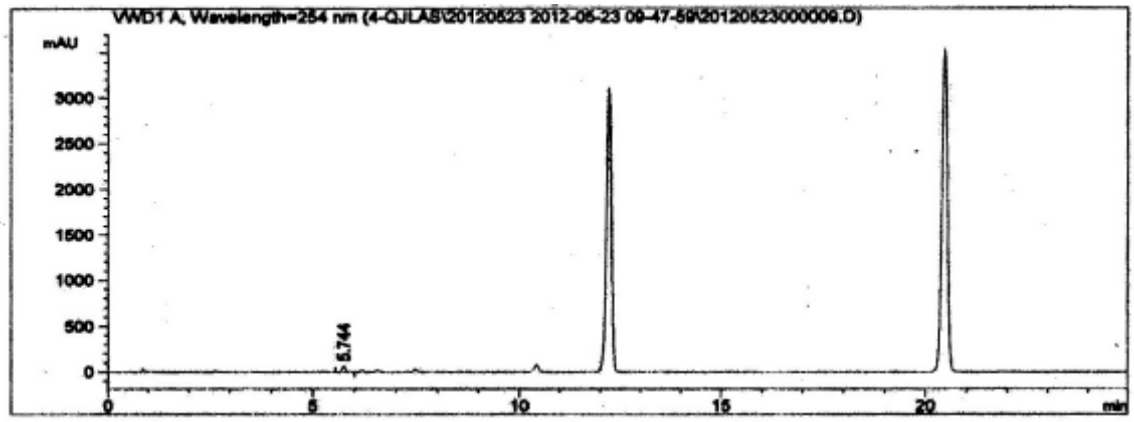
Typical HPTLC Chromatograms

These chromatograms are supplied for information only

Track assignment: 1-3) *Trigonella foenum-graecum* Seed, commercial samples; 4) USP Trigonelline Hydrochloride RS (1.5 mg/mL); 5-13) finished products (capsules and tablets); 14-16) liquid extracts.

Sample solutions:	according to the monograph
Standard solutions:	in methanol
Plate:	HPTLC, Si 60 F_{254}
Saturation time:	20 minutes
Application volume:	4 µL, as 8-mm bands
Relative Humidity:	about 33%
Temperature:	25°
Developing solvent system:	isopropyl alcohol, methanol, and water (4:1:4)
Developing distance:	6 cm
Detection:	dry, and examine under UV light at 254 nm

HPLC (4-Hydroxyisoleucine)



Representative chromatogram of Content of 4-Hydroxyisoleucine in *Trigonella foenum-graecum* Seed

This chromatogram is supplied for information only

Solution preparation: according to the monograph

Detector: UV, 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1 (similar to Kinetex™ C18 100 Å)

Column temperature: 25°±1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Solution A: 0.1% phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
20	40	60