Ginkgo

BOTANIC CHARACTERISTICS

a. Macroscopic Description

Fig. 1 Dried leaves of *Ginkgo biloba* L.

1. **General appearance:** Leaves whole, folded or fragmented, some with petioles. Lamina broadly obcuneate (fan-shaped), 2–12 cm in width and 2–9.5 cm in length from petiole to apical margin; mostly 1.5–2 times wider than long; apex sinuate, usually truncate or centrally cleft. Petiole straight, 2–8 cm in length.
2. **Surface:** Glabrous, with wrinkled appearance due to prominent dichotomous venation appearing parallel and extending from the lamina base to the apical margin.
3. **Color:** Leaves yellowish-green to greenish-brown, often browner at apical edges, and usually darker on the upper surfaces. Petioles green to greenish-brown.
4. **Odor and taste:** Slightly aromatic; slightly bitter.

b. Microscopic Description

b-1. Transverse section of leaf near petiole

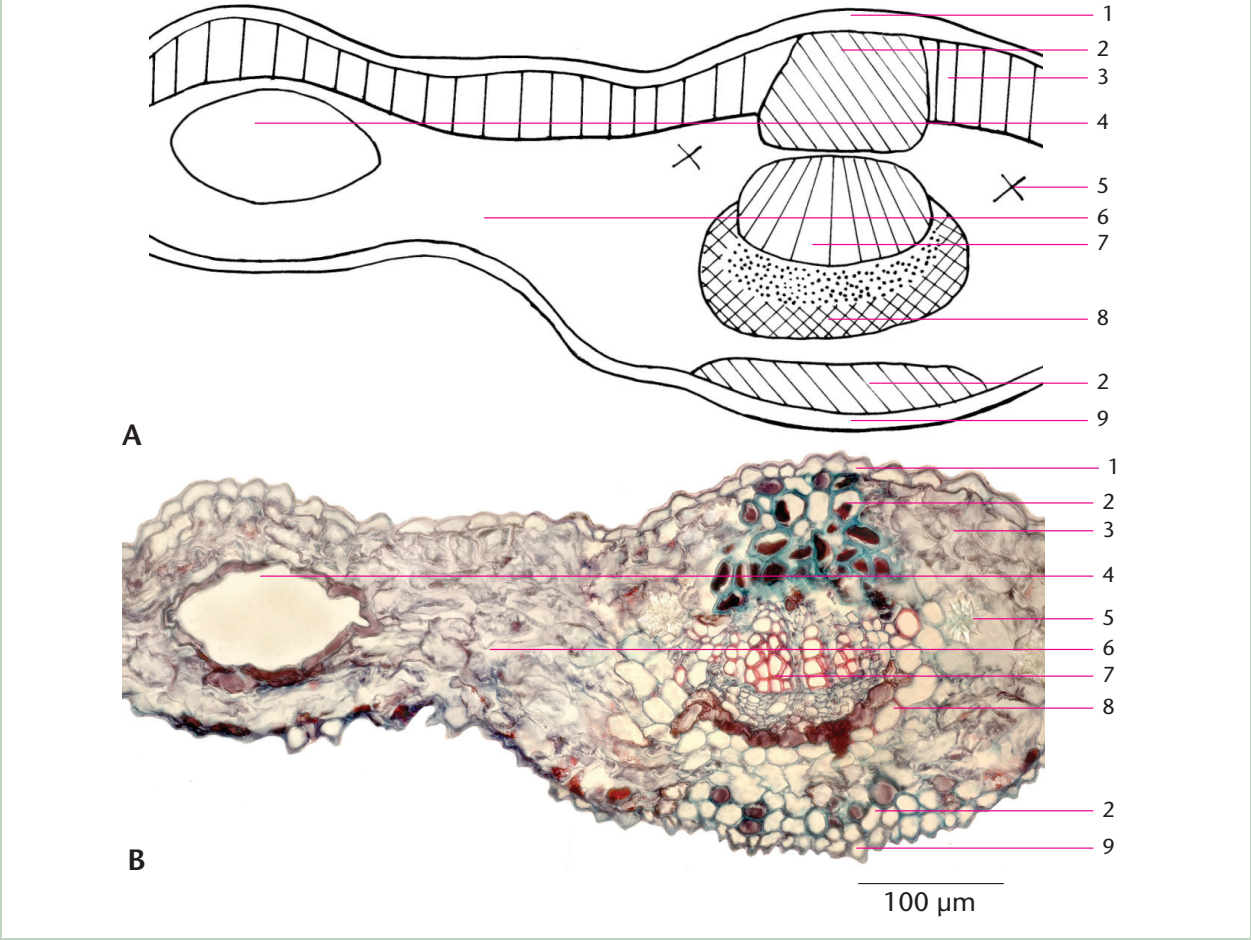


Fig. 2 Microscopic features of transverse section of *Ginkgo biloba* L. leaf near petiole

A. Sketch B. Illustration of transverse section
1. Upper epidermis 2. Collenchyma 3. Palisade tissue 4. Secretory canal
5. Clusters of calcium oxalate 6. Spongy tissue 7. Vascular bundles 8. Fibers 9. Lower epidermis

1. **Upper epidermis:** Single layer of subrounded or subsquare cells, covered with thin but marked cuticle.
2. **Collenchyma:** Arranged at the inner sides of upper and lower epidermises of midrib; only visible in mature and old leaves.
3. **Palisade tissue:** Present just underneath the upper epidermis, elongated at right angles to the surface, often irregular in appearance.
4. **Spongy tissue:** Cells of the mesophyll are smaller than the palisade cells, and separated by large intercellular spaces.
5. **Vascular bundles:** Occurring at regular intervals along the width of the lamina; with adjacent clusters of calcium oxalate; collateral, with xylem dorsal, phloem ventral, surrounded by fibers.
6. **Clusters of calcium oxalate:** Scattered among parenchymatous cells.
7. **Secretory canals:** Large and distinct, present in the mesophyll between vascular bundles.
8. **Fiber bundles:** Walls slightly thickened, surrounded vascular bundles.
9. **Lower epidermis:** Single layer of subrounded or subsquare cells, covered with thin cuticle.

b-2. Powder: Greenish-brown.

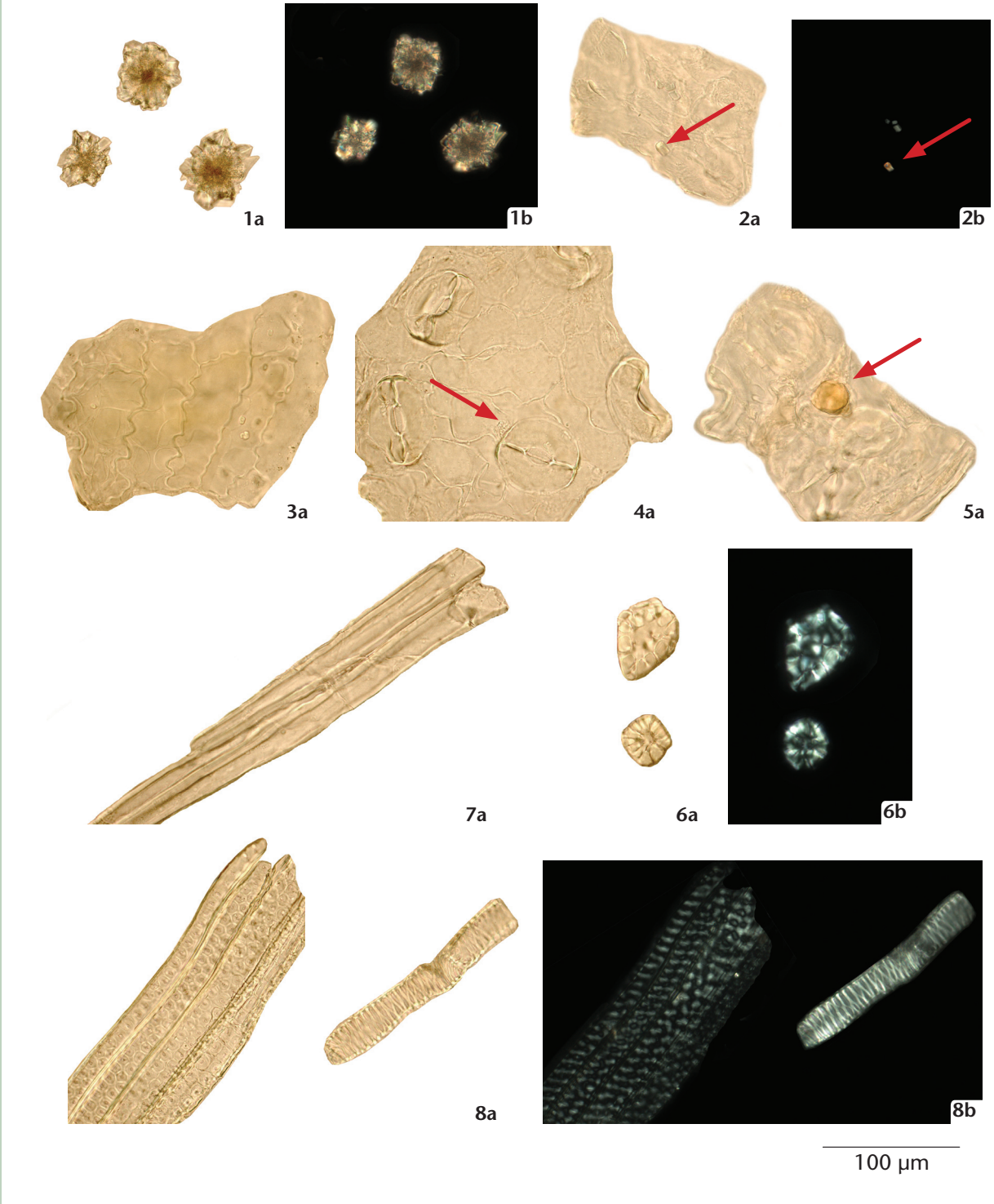


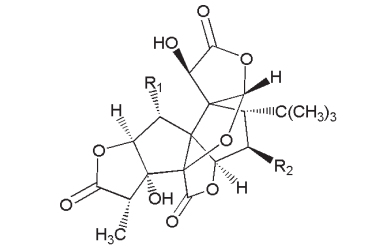
Fig. 3 Microscopic features of powder of *Ginkgo biloba* L. leaf

a. Features under the light microscope b. Features under the polarized light microscope
1. Clusters of calcium oxalate 2. Prisms of calcium oxalate 3. Oil droplets 4. Upper epidermis of leaf
5. Lower epidermis of leaf (stomata →) 6. Fibers 7. Stone cells 8. Tracheids

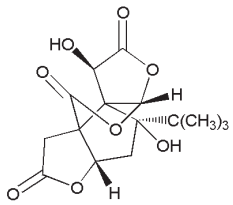
1. **Clusters of calcium oxalate:** Abundant, scattered or associated with vessels, (5)-15 to 100-(120) μm in diameter; bright polychrome when observed under the polarized light microscope.
2. **Prisms of calcium oxalate:** Scattered or present in parenchymatous cells; polychrome or bright white when observed under the polarized light microscope.
3. **Upper epidermis:** Subrectangular cells with wavy anticlinal walls.
4. **Lower epidermis:** Subrectangular or subrounded cells with slightly wavy anticlinal walls; stomata anisocytic, with guard cells sometimes sunken with respect to adjacent epidermal cells.
5. **Oil droplets:** Present in parenchymatous cells, yellowish.
6. **Fibers:** Long fusiform, mostly broken, 10–25 μm in diameter.
7. **Stone cells:** Usually found in petioles, subrectangular, subrounded or subsquare, 20–45 μm in diameter; bright yellowish-white when observed under the polarized light microscope.
8. **Tracheids:** Mainly bordered-pitted and reticulate, 7–30 μm in diameter; bright white when observed under the polarized light microscope.

CHEMICAL CHARACTERISTICS

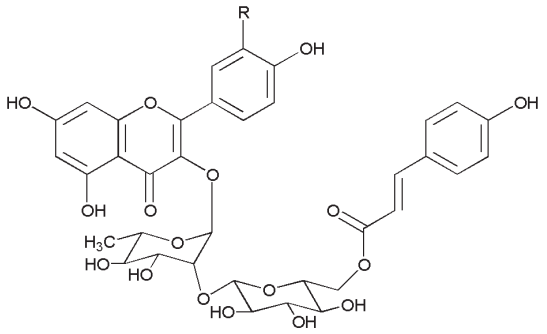
a. Chemical Structures



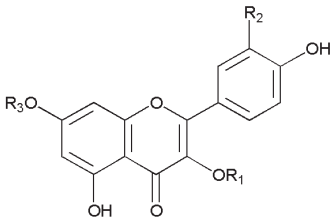
	R_1	R_2
Ginkgolide A	H	H
Ginkgolide B	OH	H
Ginkgolide C	OH	OH
Ginkgolide J	H	OH



Bilobalide



Kaempferol-3-O-(6'''-trans-p-coumaroyl-2''-glucosyl)rhamnoside : R=H
Quercetin-3-O-(6'''-trans-p-coumaroyl-2''-glucosyl)rhamnoside : R=OH



	R_1	R_2	R_3
Isorhamnetin	H	OCH_3	H
Kaempferol	H	H	H
Quercetin	H	OH	H
Kaempferol-3-*O*-glucoside	Glc	H	H
Kaempferol-7-*O*-glucoside	H	H	Glc
Kaempferol-3-*O*-rutinoside	Rut (L-Rha(α1-6)Glc)	H	H
Quercetin-3-*O*-glucoside	Glc	OH	H
Quercitrin (Quercetin-3-*O*-rhamnoside)	Rha	OH	H
Rutin (Quercetin-3-*O*-rutinoside)	Rut (L-Rha(α1-6)Glc)	OH	H
Isorhamnetin-3-*O*-glucoside	Glc	OCH_3	H
Isorhamnetin-3-*O*-rutinoside	Rut (L-Rha(α1-6)Glc)	OCH_3	H

Fig. 4 Constituents of *Ginkgo biloba* L. leaf

b. Thin Layer Chromatography

Test for flavonoids

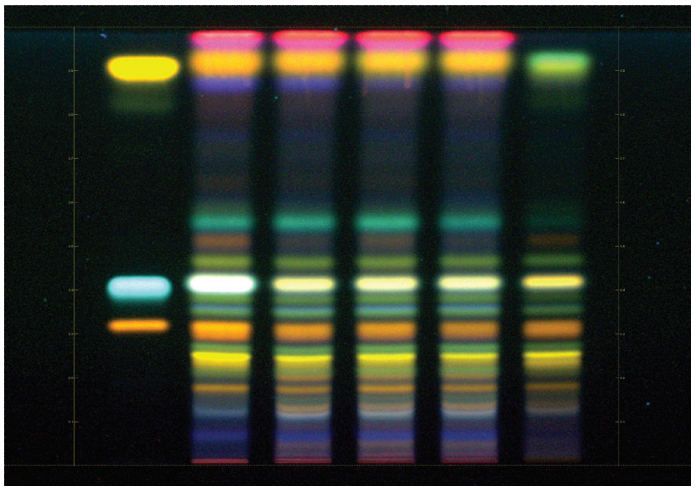


Fig. 5 Typical HPTLC chromatograms

Track assignment: 1) USP Rutin RS 0.6 mg/mL, USP Chlorogenic RS 0.2 mg/mL, and USP Quercetin RS (with increasing R_F); 2) ginkgo leaves (commercial sample); 3) ginkgo leaves (wild crafted); 4) ginkgo leaves, powder (commercial sample A); 5) ginkgo leaves, powder (commercial sample B); 6) ginkgo leaves, tincture (commercial sample)

Sample solutions:	according to the monograph
Standard solution:	in methanol
Plate:	HPTLC, Si 60 F_{254}, 5-µm
Developing solvent:	ethyl acetate, water, formic acid and glacial acetic acid (100:26:11:11)
Saturation time:	20 min, with filter paper
Relative humidity:	37%
Temperature:	23°
Application volume:	5 µL
Detection:	heat at 100° for 5 min, derivatize with reagent (a) then reagent (b), dry, and examine under UV light at 366 nm
Derivatization reagent:	a) 2-aminoethyl diphenylborniate in ethyl acetate (5 mg/mL) b) polyethylene glycol 400 in methylene chloride (50 mg/mL)

Test for terpene lactones

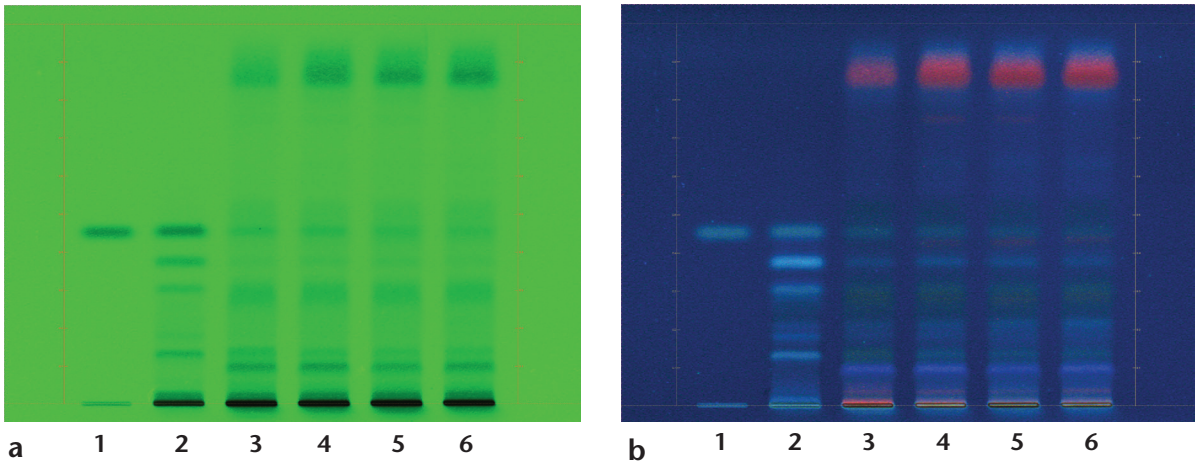


Fig. 6 Typical HPTLC chromatograms

Track assignment: 1) bilobalide (commercial sample), 1 mg/mL; 2) USP Ginkgo Terpene Lactones RS, 10 mg/mL; 3) ginkgo leaves (commercial sample); 4) ginkgo leaves (wild crafted); 5) ginkgo leaves, powder (commercial sample A); 6) ginkgo leaves, powder (commercial sample B)

Test Solution:	according to the monograph
Standard solution:	in methanol
Plate:	HPTLC, Si 60 F_{254}, 5-µm, impregnated with a solution of sodium acetate in methanol (4 g per 100 mL) for 2 sec.
Developing solvent:	toluene, ethyl acetate, acetone, and methanol (20:10:10:1.2)
Saturation time:	20 min, with filter paper
Relative humidity:	37%
Temperature:	23°
Application volume:	5 µL
Detection:	derivatize, heat at 180° for 10 min, cool, and examine under UV light at 254 nm (a) and at 366 nm (b)
Derivatization reagent:	acetic anhydride

Other non-USP thin layer chromatography – Test for flavonoids

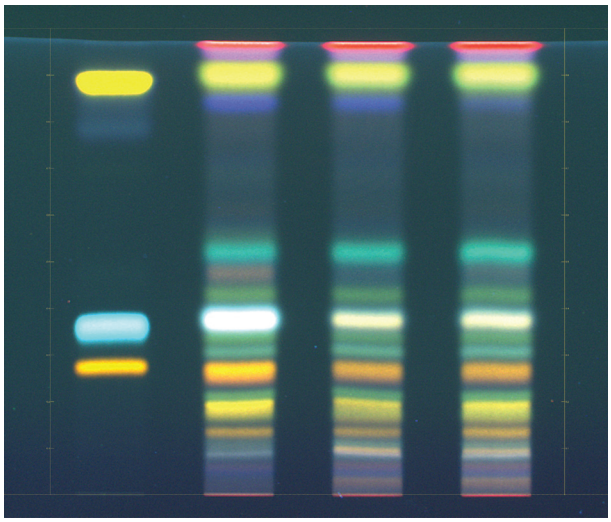


Fig. 7 Typical TLC chromatograms

Track assignment: 1) USP Rutin RS 0.6 mg/mL, USP Chlorogenic RS 0.2 mg/mL, and USP Quercetin RS (with increasing R_F); 2) ginkgo leaves (commercial sample); 3) ginkgo leaves (wild crafted); 4) ginkgo leaves, powder (commercial sample)

Sample solutions:	0.2 g sample refluxed in 10 mL methanol for 10 min, cool, filter, and concentrate the filtrate to half its volume
Standard solution:	in methanol
Plate:	TLC, Si 60 F_{254}
Developing solvent:	ethyl acetate, water, anhydrous formic acid and glacial acetic acid (67.5:17.5:7.5:7.5)
Saturation time:	20 min, with filter paper
Relative humidity:	57%
Temperature:	23°
Application volume:	20 µL
Detection:	heat at 100° for 5 min, derivatize with reagent (a) then reagent (b), dry, and examine under UV light at 366 nm
Derivatization reagent:	a) 2-aminoethyl diphenylborniate in ethyl acetate (5 mg/mL) b) polyethylene glycol 400 in methylene chloride (50 mg/mL)

c. High Performance Liquid Chromatography

Content of flavonol glycosides

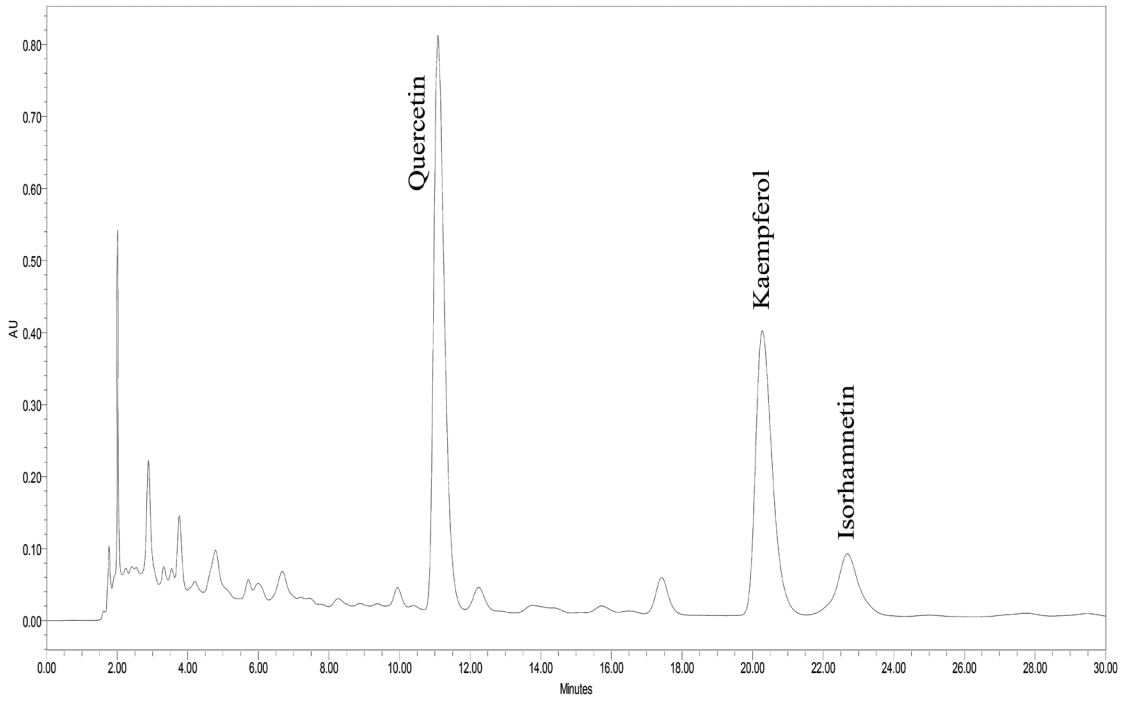


Fig. 8 Typical chromatogram of Sample solution

Solutions preparation:	according to the monograph
Column:	L1, 25-cm × 4.6-mm, 5-µm, Prodigy ODS(3), Phenomenex
Mobile phase:	methanol, water, and phosphoric acid (100:100:1)
Elution:	isocratic
Flow rate:	1.5 mL/min
Temperature:	25°
Injection volume:	20 µL
Detection:	UV, 270 nm

Content of terpene lactones

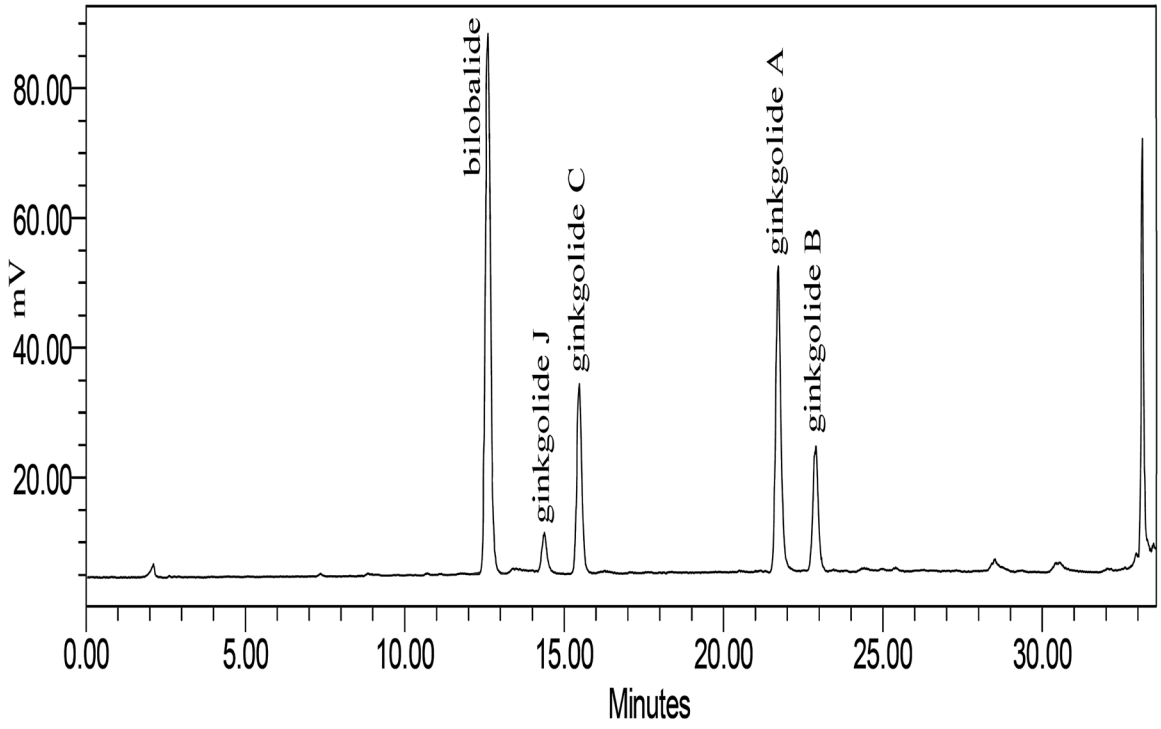


Fig. 9 Typical chromatogram of USP Ginkgo Terpene Lactones RS

Solutions preparation: according to the monograph

Column: L1, 25-cm × 4.6-mm, 5-µm, Prodigy ODS(3), Phenomenex

Mobile phase: water (Solution A) and methanol (Solution B)

Elution: gradient program, see below

Flow rate: 1 mL/min

Temperature: 25°

Injection volume: 15 µL

Detection: ELSD, Sedex Model 75, Sedere - France, drift tube temperature 52°, nitrogen pressure 44.1 psi

Time (min)	Solution A (%)	Solution B (%)	Elution
0–23	75–52	25–48	Linear gradient
23–28	52	48	Isocratic
28–30	52–25	48–75	Linear gradient
30–35	25–10	75–90	Linear gradient
35–40	10–75	90–25	Linear gradient
40–50	75	25	Isocratic

Limit of ginkgolic acids

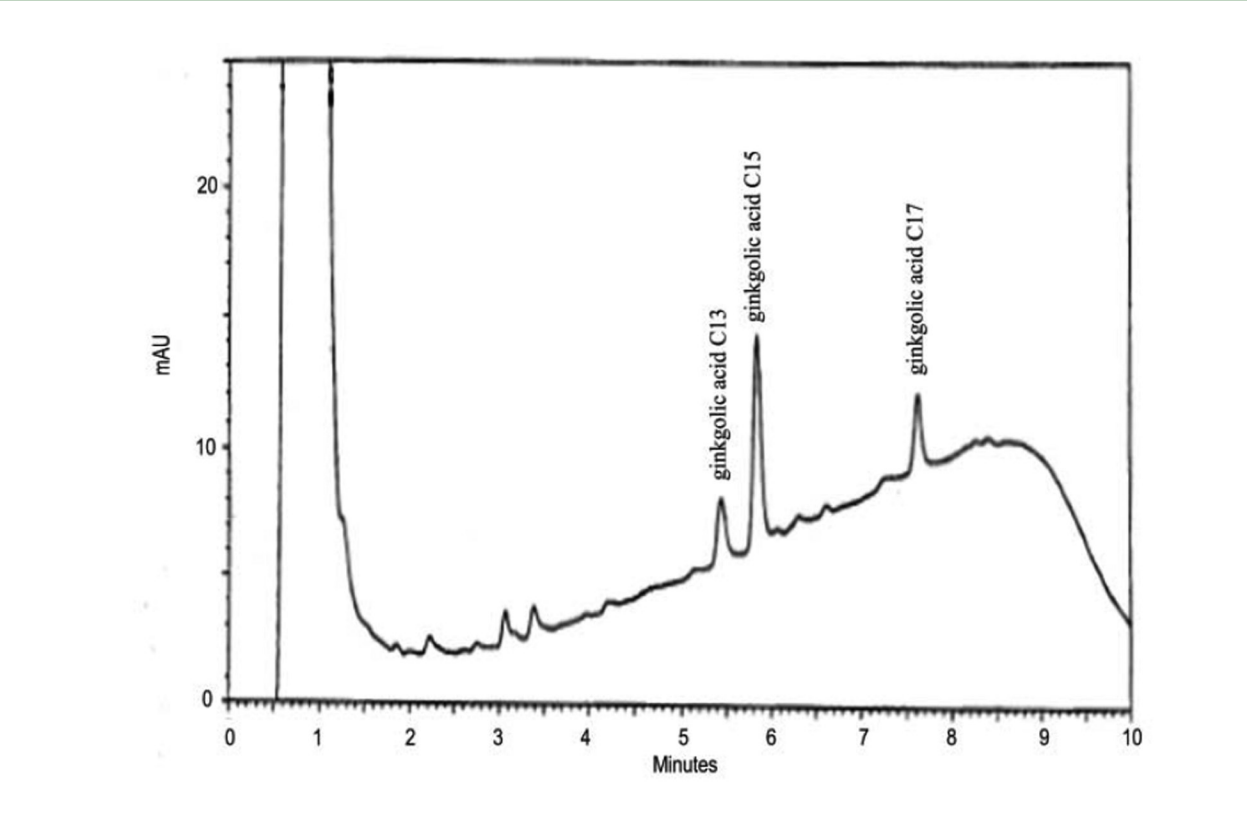


Fig. 10 Typical chromatogram of USP Ginkgolic Acids RS

Solutions preparation: according to the monograph

Column: L7, 5-cm × 4.6-mm, 3.5-µm, Zorbax XDB-C8, Agilent

Mobile phase: 0.01 % phosphoric acid in water (Solution A) and 0.01% phosphoric acid in acetonitrile (Solution B)

Elution: gradient program, see below

Flow rate: 1 mL/min

Temperature: 35°

Injection volume: 100 µL

Detection: UV, 210 nm

Time (min)	Solution A (%)	Solution B (%)	Elution
0–6	25–10	75–90	Linear gradient
6–7	10	90	Isocratic
7–8	10–25	90–75	Linear gradient
8–10	25	75	Isocratic