Morus alba L. – Leaf

1. Scope

This method identifies dried leaves of *Morus alba L.* by HPTLC fingerprint and discriminates twigs of *Morus alba* L., leaves of *Morus nigra* L, and leaves of *Ribes nigrum* L. .

2. Authors and adoptions

Ilona Trettin, HPTLC Association
Under evaluation by Ph. Eur.

3. Procedure

Test solution	Mix 5.00 g of powdered sample with 10.0 mL of methanol and sonicate for 10 minutes, then centrifuge the mixture. Use the supernatant as test solution.
Reference solutions	SST: Universal HPTLC mix (UHM) - prepared in house 0.15 mg/mL of rutin 0.40 mg/mL of chlorogenic acid 0.10 mg/mL of hyperoside and isoquercitrin in methanol.
Stationary phase	HPTLC Si 60 F_{254} (Merck)
Application	15 tracks, band length 8.0 mm, track distance 11.4 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volume 2.0 µL of reference and test solutions.
Developing solvent	Ethyl acetate, methyl ethyl ketone, formic acid, water 50:30:10:10 *(V/V)*
Developing distance	70 mm from lower edge of the plate
Saturation time	20 min, with a saturation pad
Relative humidity	33%, saturated $MgCl_2$
Temperature	22 ± 5°C
Derivatization reagent A	Natural poducts reagent (NP reagent - for spraying Preparation: Dissolve 1.0 g of 2-aminoethyl dihenylborinate in 100.0 mL of methanol. Use: Heat at 100°C for 3 min, let the plate cool down, spray (Derivatizer: 3.0 mL, green nozzle, spraying level 3) with *Derivatization reagent A*, dry for 2 min.
Derivatization reagent B	Anisaldehyde reagent Preparation: Slowly and carefully mix 170.0 mL of ice-cooled methanol with 20.0 mL of acetic acid and 10.0 mL of sulfuric acid. Allow the mixture to cool to room temperature, then add 1.0 mL of anisaldehyde (*p*-methoxy benzaldehyde). Use: Derivatize Derivatizer: 3.0 mL, blue nozzle, spraying level 3) with *Derivatization reagent B*, heat at 100°C for 3 min, let cool down.
Detection	A) Underivatized, shortwave UV (254 nm) B) Underivatized, longwave UV (350 nm broadband) C) Derivatized A, longwave UV (350 nm broadband) D) Derivatized A, white light RT) E) Derivatized A+B, longwave UV (350 nm broadband) F) Derivatized A+B, white light RT

4. Results

Note: These chromatographic fingerprints are representative of the samples used in this analysis. Fingerprints obtained may vary from sample to sample. Analysts must validate the most appropriate fingerprint for their identity standard.

System suitability test – Detection A)

- Quenching zone at R_F ~ 0.12
- Quenching zone at R_F ~ 0.49
- Quenching zone at R_F ~ 0.84

Specific markers (Detection C)

- rutin: orange brown zone at R_F ~ 0.31
- chlorogenic acid: light blue zone at R_F ~ 0.42
- hyperoside: orange brown zone at R_F ~ 0.50
- isoquercitrin: orange brown zone at R_F ~ 0.55

Different origin(s)

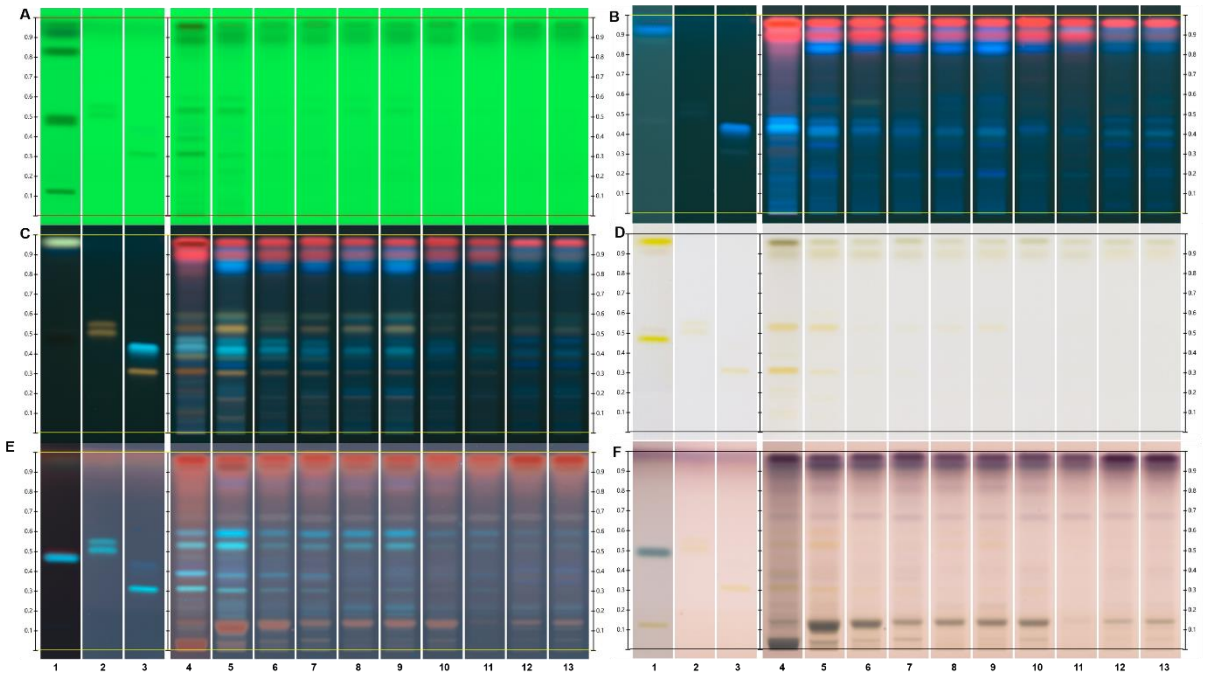
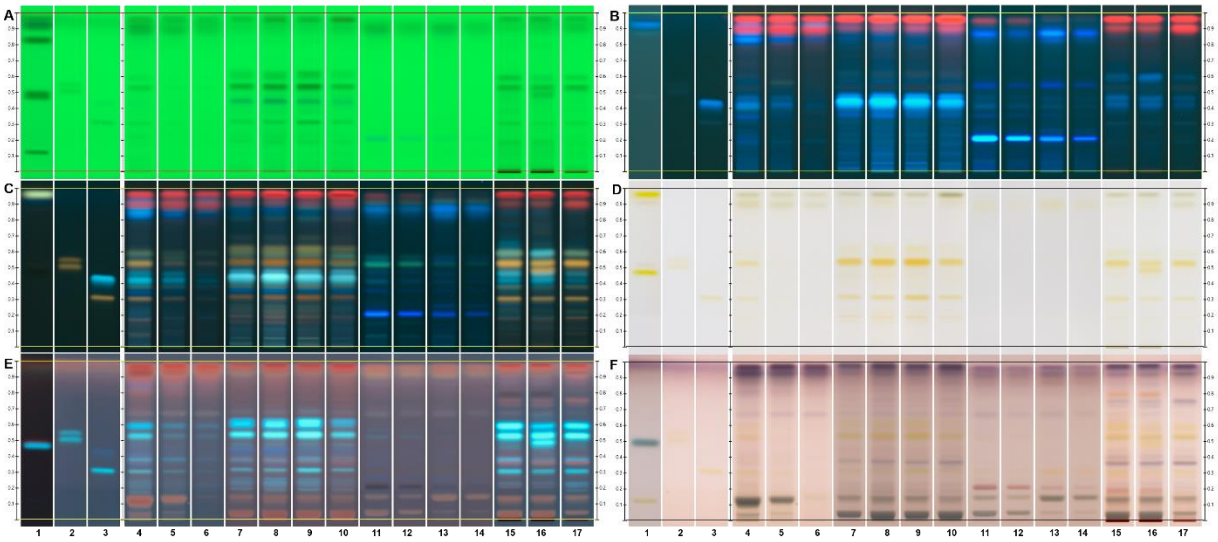


*Figure 1: HPTLC fingerprints in shortwave UV (**A**) and longwave UV (**B**) prior to derivatization; longwave UV (**C**) and white light RT (**D**) after derivatization A; longwave UV (**E**) and white light RT (**F**) after derivatization B,*

Track	Sample	Origin
1	UHM	-
2	Hyperoside, isoquercitrin with increasing R_F	-
3	Rutin, chlorogenic acid with increasing R_F	-
4- 13	*Morus alba* – powdered leaf	China (CN)

Related herbal drugs



*HPTLC fingerprints in shortwave UV (**A**) and longwave UV (**B**) prior to derivatization; longwave UV (**C**) and white light RT (**D**) after derivatization A; longwave UV (**E**) and white light RT (**F**) after derivatization B,*

Track	Sample	Origin
1	UHM	-
2	Hyperoside, isoquercitrin with increasing R_F	-
3	Rutin, chlorogenic acid with increasing R_F	-
4-6	*Morus alba* – powdered leaves	China (CN)
7-10	*Morus nigra* – powdered leaves (same preparation as test solution)	Germany (DE)
11-14	*Morus alba* – powdered twigs (same preparation as test solution)	China (CN)
15-17	*Ribes nigrum* – powdered leaves (same preparation as test solution)	Switzerland (CH)

Version	Revision history	Released by
1	Created by: ER/30 Aug 2022	TD/30 Aug 2022