Cape Aloes (*Aloe ferox*)

1. Scope

This method identifies the concentrated and dried juice of the leaves of Cape Aloes (*Aloe ferox* Mill.) by HPTLC fingerprint and detects adulteration with the concentrated and dried juice of the leaves of Barbados Aloes (*Aloe barbadensis* Mill.).

2. Source of method

Modified from Ph.Eur. 9.2: heating at 100°C for 3 min instead of heating at 110°C for 5 min

3. Procedure

Test solution:	To 0.25 g of the powdered sample add 20 mL of methanol and heat to boiling in a water-bath. Shake for two minutes, centrifuge or decant the solution and use the supernatant as test solution. Store at about 4 °C and use within 24 h.
Reference solution:	Dissolve 2 mg of barbaloin (=aloin) and 2 mg aloe-emodin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, methanol, water 100:17:13 (v/v/v)
Development:	- Saturated chamber - Developing distance 70 mm from lower edge - Relative humidity 33%
Derivatization reagent:	KOH reagent Preparation: 20 g of potassium hydroxide are dissolved in 200 mL of methanol (prepare in an ice-bath). Use: Dip (time 0, speed 5), heat at 110°C for 5 minutes
Documentation:	1.) Developed plate, UV 366 nm 2.) KOH reagent, white light RT

4. Results

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

Fig. 1) Developed plate, UV 366 nm

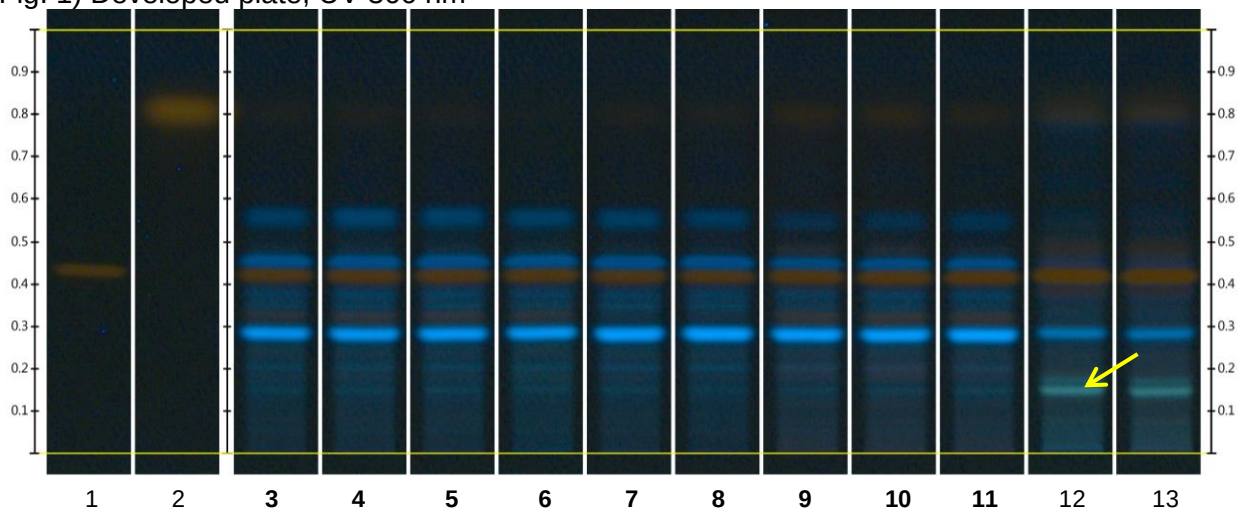
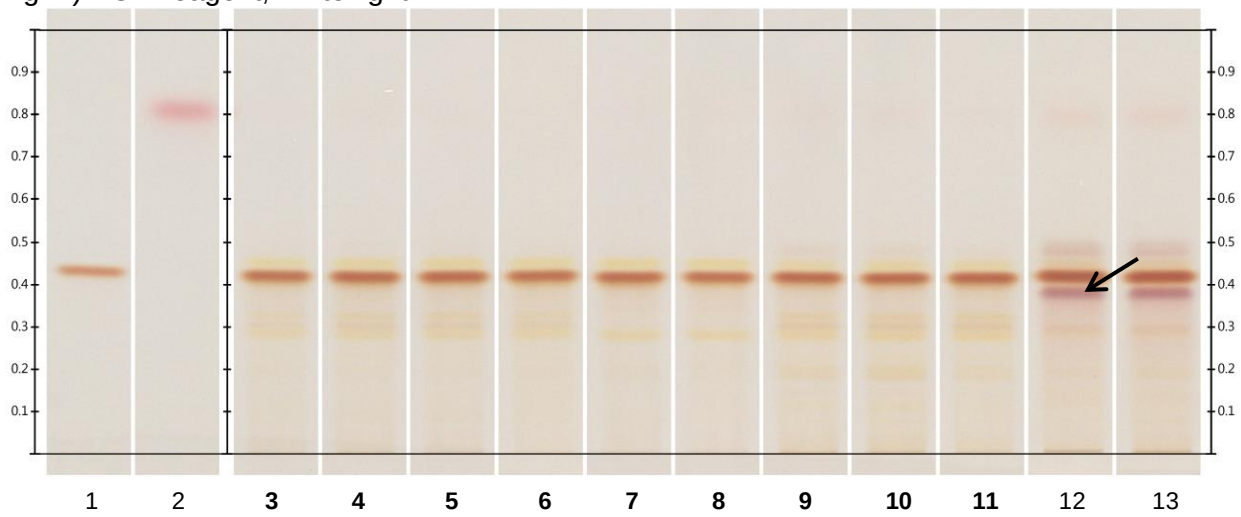


Fig. 2) KOH reagent, white light RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Barbaloin	**8**	**2 µL**	**Cape Aloes 6**
2	2 µL	Aloe-emodin	**9**	**2 µL**	**(Cape) Aloes dry extract (aqueous) 1**
3	**2 µL**	**Cape Aloes 1**	**10**	**2 µL**	**(Cape) Aloes dry extract (aqueous) 2**
4	**2 µL**	**Cape Aloes 2**	**11**	**2 µL**	**(Cape) Aloes dry extract (aqueous) 3**
5	**2 µL**	**Cape Aloes 3**	12	2 µL	Barbados Aloes
6	**2 µL**	**Cape Aloes 4**	13	2 µL	Barbados Aloes
7	**2 µL**	**Cape Aloes 5**			

System suitability test (under UV 366 nm)
Barbaloin: an orange fluorescent zone at R_F ~ 0.42
Aloe emodin: a yellow fluorescent zone at R_F ~ 0.82

Identification
Compare result with reference images. The fingerprint of the test solution prepared from a sample is similar to those prepared from corresponding botanical reference samples (marked in bold). Additional weak zones may be present.

Under UV 366 nm the chromatogram obtained with the test solution shows an orange fluorescent zone corresponding to reference barbaloin and below it a bluish-white fluorescent zone. Above the zone due to barbaloin there are two blue fluorescent zones.

After derivatization with KOH reagent, under white light RT, the chromatogram obtained with the test solution shows a brown zone corresponding to reference substance barbaloin.

Test for adulteration with Barbados Aloes
Under UV 366 nm the chromatogram obtained with the test solution shows no prominent bluish-green fluorescent zone (yellow arrow) below the bluish-white fluorescent zone in the lower third.

After derivatization with KOH reagent, under white light RT, the chromatogram obtained with the test solution shows no violet zone just below the zone due to barbaloin (black arrow).