

## Identification of wintergreen oil by HPTLC and discrimination of pure (synthetic) methyl salicylate

A-114.1



### Keywords

*Gaultheria procumbens* L., adulteration, essential oil, natural

### Introduction

Wintergreen is a tiny dense shrub of about fifteen centimeters high. This shrub is native to North America, Australia, East Asia, and the Himalayas [1]. Wintergreen essential oil is usually obtained by steam distillation of the leaves of the plant. It is mainly composed of methyl salicylate (97-99%),  $\alpha$ -terpineol (0.08%), 1,8-cineole (0.02%) [2].

### Scope

This method is suitable for the identification of “natural” wintergreen essential oil under standard HPTLC conditions and also allows the discrimination of “pure” synthetic methyl salicylate.

### Recommended devices

Automatic TLC Sampler (ATS 4), Automatic Developing Chamber (ADC 2), TLC Visualizer, *visionCATS* software, Derivatizer

### Samples

50  $\mu$ L of wintergreen essential oil is dissolved in 1 mL of toluene

NOTE: The presented results are to be regarded as examples only!

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## Standards

**SST:** Dissolve 2.5  $\mu\text{L}$  of isoeugenol and 5 mg of isoeugenyl acetate in 10.0 mL of toluene.

**Specific reference:** Dissolve 10  $\mu\text{L}$  of  $\alpha$ -terpineol, 10  $\mu\text{L}$  of 1.8-cineole and 20  $\mu\text{L}$  of methyl salicylate in 10.0 mL of toluene

## Chromatography

Stationary phase	HPTLC Si 60 F <sub>254</sub> , 20 x 10 cm (Merck)
Sample application	Bandwise application, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume of 2 $\mu\text{L}$ of standard, and 5 $\mu\text{L}$ of each sample
Developing solvent	Toluene, ethyl acetate 95:5 (v/v)
Development	In the ADC 2 with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride (MgCl <sub>2</sub> ).
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the ADC 2
Detection A:	With the TLC Visualizer under UV 254 nm
Derivatization reagent	To 170 mL of cold methanol 20 mL of acetic acid and 10 mL of sulfuric acid are added and mixed well. After cooling to room temperature 1 mL of anisaldehyde (p-methoxy benzaldehyde) are added to the mixture
Detection B:	Derivatize the plate with the Derivatization reagent (nozzle blue / level 3, 3 mL) and heat the plate at 100 °C for 3 min. Examine the plate under white light with the TLC Visualizer

## Results

### System Suitability Test (SST) under UV 254 nm:

Isoeugenyl acetate: a faint purple zone at  $R_F \sim 0.35$

Isoeugenol: a faint purple zone at  $R_F \sim 0.40$

### Specific reference under UV 254 nm prior to derivatization:

Methyl salicylate: a quenching zone at  $R_F \sim 0.61$  (detection A)

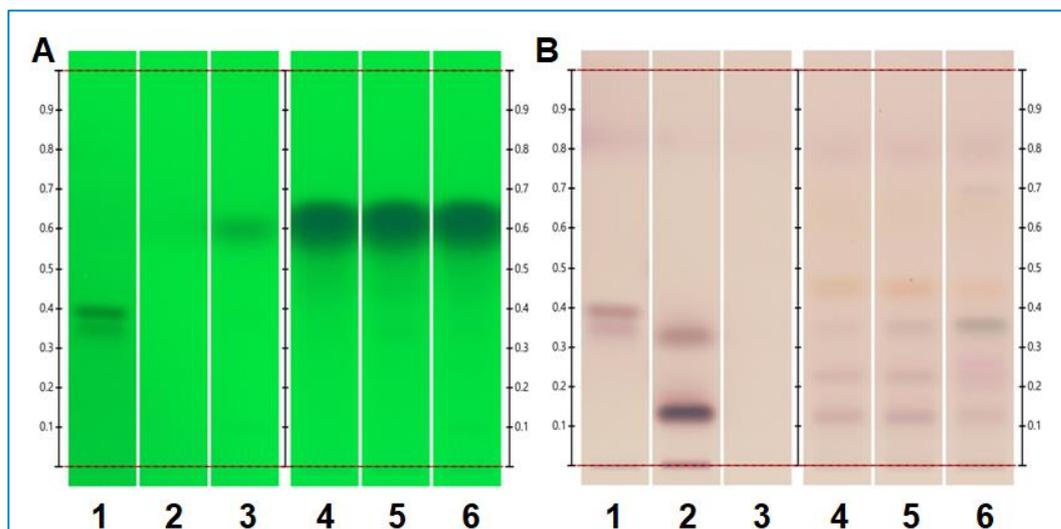
$\alpha$ -terpineol: a purple zone at  $R_F \sim 0.14$  (detection B)

1.8-cineole: a purple zone at  $R_F \sim 0.33$  (detection B)

The sequence of the zones present in the chromatograms of wintergreen oil is shown below. Other faint zones may be seen in the chromatogram of the test solution.

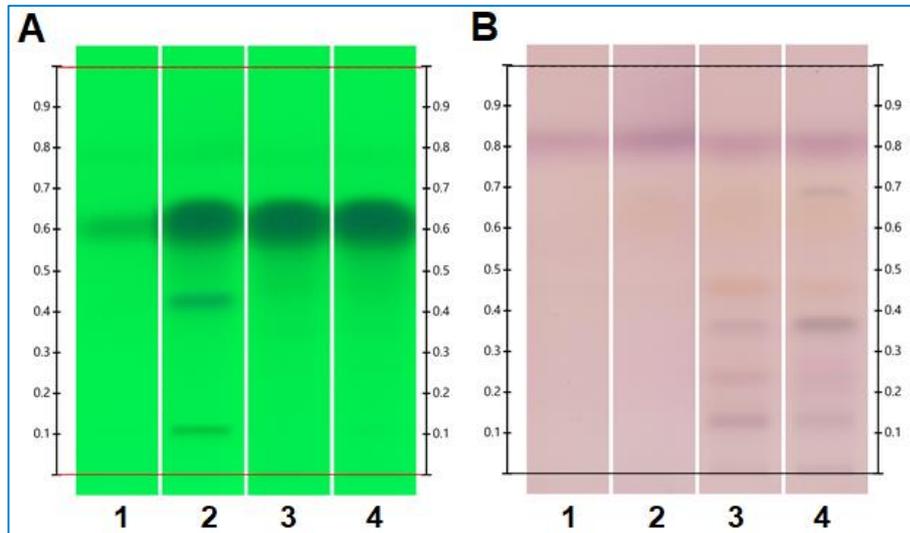
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A: HPTLC under UV 254 nm prior to derivatization; B: HPTLC under white light WRT after derivatization. Track 1: isoeugenyl acetate (0.5 mg/mL), isoeugenol (0.25 µL/mL) (with increasing  $R_F$ ), track 2:  $\alpha$ -terpineol (1.0 µL/mL), 1,8-cineole (1.0 µL/mL) (with increasing  $R_F$ ), track 3: methyl salicylate (2 µL/mL), tracks 4-6: test solution of wintergreen oils (50 µL/mL)

For the discrimination with pure (synthetic) methyl salicylate, the standard (purity of 99%) was prepared at the same concentration as the essential oil (50 µL/mL). Under UV 254 nm prior to derivatization other faint zones of impurities may be seen, but under white light after derivatization no other zones are observed.



A: HPTLC under UV 254 nm prior to derivatization; B: HPTLC under white light WRT after derivatization. Track 1: methyl salicylate (2 µL/mL), track 2: methyl salicylate (50 µL/mL), tracks 3-4: test solution of wintergreen oils (50 µL/mL)

## Literature

- [1] Fernandez X., Chemat F., Do TKT.: Les huiles essentielles. Vertus et application. Ed. Vuibert, Paris, 2012.  
 [2] Norm NF ISO 11024: wintergreen essential oil

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