

Biological activity of berberine containing drugs by HPTLC-Bioluminescence screening

F-34



Scope:

This method illustrates the possibilities of activity related detection using bioluminescent bacteria *Vibrio fischeri*. Specific biological or toxicological properties of plant constituents, which have been separated by HPTLC, can be revealed with this detection.

Sample:

Berberine containing drug: e.g. *Mahonia*, *Coptis*, *Phellodendron*, *Tinospora* etc.
0.3 g powdered raw material is mixed with 10 mL of methanol, sonicated for 10 min, and centrifuged. The supernatant is used as test solution.

Standards (optional):

1 mg each of palmatine hydrochloride and berberine hydrochloride are individually dissolved in 5 mL of methanol.

Bioluminex™ assay:

Using the Bioluminex™ kit (ChromaDex Analytics, Inc., Boulder, CO) from lyophilized *Vibrio fischeri* a culture is grown overnight in Bioluminex medium. Directly before assay Bioluminex buffer A is added to the fully luminescent bacteria.

Chromatographic conditions:

Stationary phase:	HPTLC plates silica gel 60 F ₂₅₄ (Merck), 10x10 cm or 20x10 cm.
Mobile Phase:	toluene, ethyl acetate, methanol, isopropanol, water (60:30:20:15:3)
Sample application:	1 µL test and standard solution are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.
Development:	10x10 cm or 20x10 cm Twin Trough Chamber, saturated for 20 min with concentrated ammonia in one trough and 5 mL (respectively 10 mL) developing solvent in the other trough, developing distance 70 mm from lower

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APPLICATION NOTES

edge of plate. The plate is then dried with a hair dryer (cold air) for 5 min.

Detection:

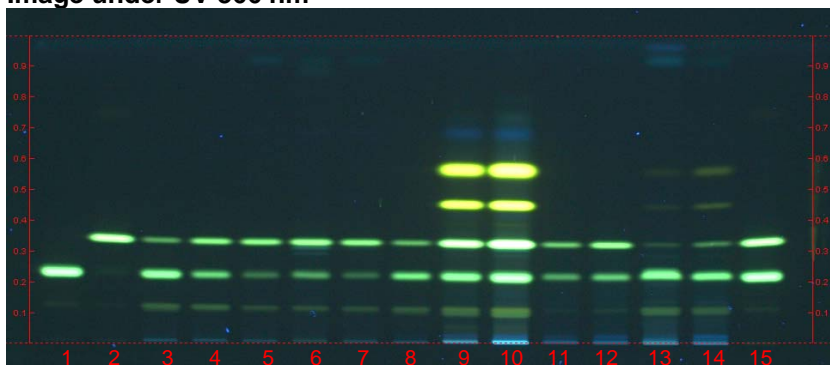
a) Examination under UV 366 nm.

b) Bioluminex™ assay: The plate is immersed for 2 s into luminescent bacteria. Examination (exposure time 55 s, incubation time 3 min) with BioLuminizer™.

Results:

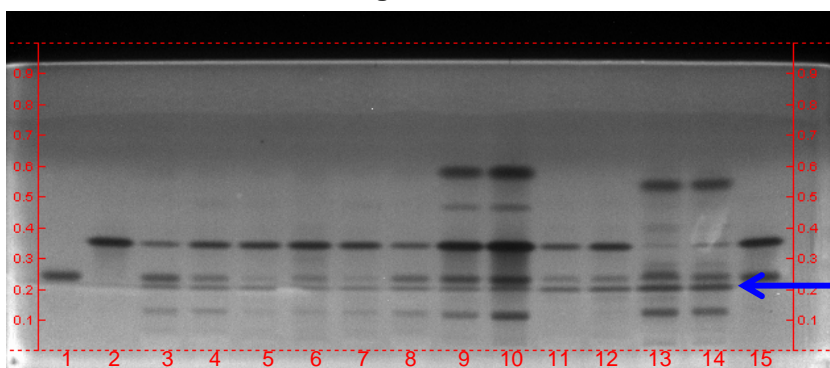
Compare to the chromatograms below:

Image under UV 366 nm



- 1: Palmatine
- 2: Berberine
- 3-8: *Mahonia sp.*
- 9-10: *Coptis chinensis*
- 11-12: *Phellodendron chinensis*
- 13-14: *Tinospora sp.*
- 15: Palmatine, Berberine

HPTLC-bioluminescence image



Berberine alkaloids can be easily detected by their green to yellow fluorescence under UV 366 nm. They are considered to be the active ingredients of many medicinal plants and their biological activity is revealed by the Bioluminex™ assay. The blue arrow indicates an additional compound which cannot be seen under UV 366 nm.

References:

- Method adapted from Pharmacopoeia of the P.R. of China, 2005
- ChromaDex Bioluminex™ assay: <http://www.bioluminex.com/>
- CAMAG BioLuminizer™: <http://www.camag.com/v/products/evaluation/bioluminizer.html>

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