Analyze for the needle not the haystack!

ChromaDex™

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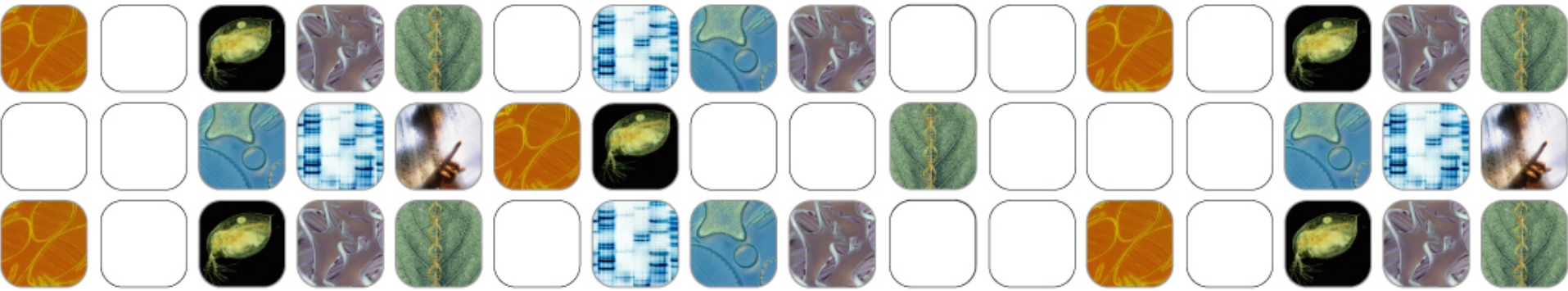
Technology Invented By Bayer

bioluminex

Rapid Screening of Complex Mixtures by TLC-Bioluminescence.



International Symposium for Thin Layer Chromatography, Berlin, Germany, October 9th-11th, 2006, Manufacture's Section.

ChromaDex™ Company Overview

- ChromaDex™ is a Life Sciences business that is privately owned and independently run.
- ChromaDex™ was established in 1999 to become the market leader in the creation and supply of botanical reference standards along with related phytochemical products and services.
- ChromaDex™ is a supplier of both analytical reference materials and contract analytical services covering the natural products industry (herbal products, spices, nutraceuticals).
- In 2005, ranked #305 of America's 500 Fastest Growing Private Companies by "Inc.".

Bioluminex Assay History

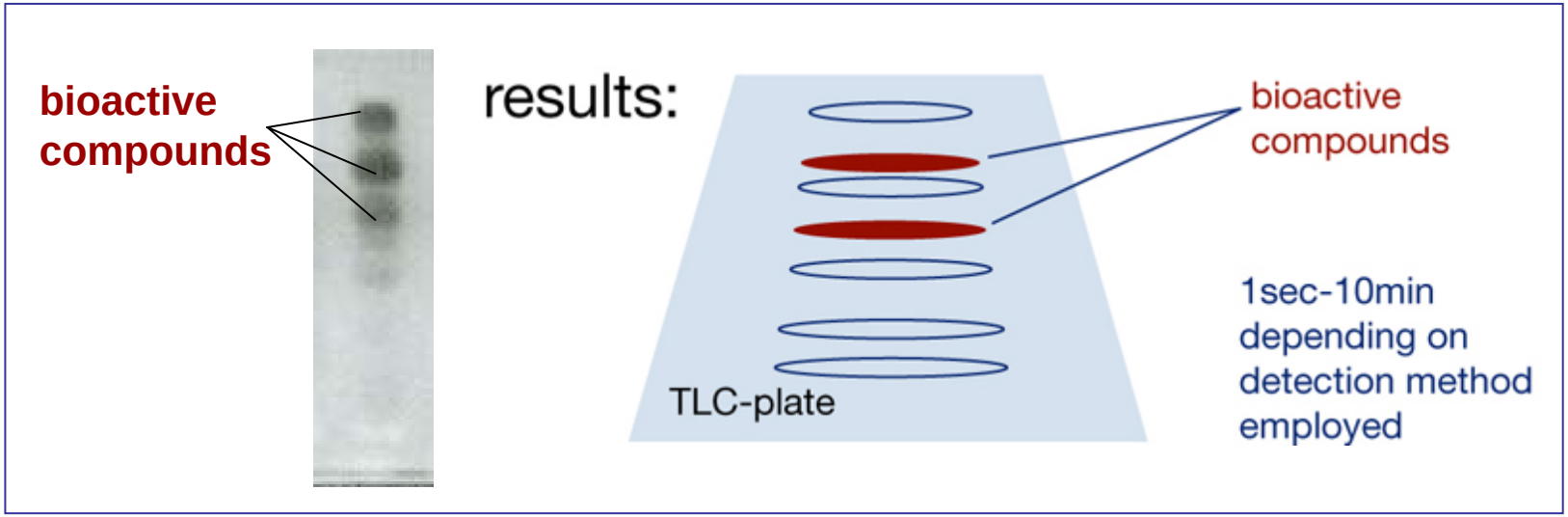
- Licensed the Bioluminex Technology from Bayer Industry Services, Leverkusen, Germany in 2001
- In 2003, ChromaDex received a FDA Grant FD-U-002514-0, “Rapid Screening of Foods for Toxins by TLC-Bioluminescence.”
- In early 2006, Chromadex filed a Patent based on Bioluminex improvements.
- Commercial Bioluminex Kit introduced to the market in early 2006.

Bioluminex Assay

- Innovative application of existing technology.
  - Thin-layer chromatography (TLC) separates complex mixtures.
  - Biosensor properties of the bioluminescent bacteria, *Vibrio fischeri*
- Pesticides (fungicides, insecticides, herbicides), heavy metals, organic pollutants, pharmaceuticals, and mycotoxins.
- Dietary supplements and natural products, food stuff, beverages, and waste water.
- 17 complex samples with standard and controls can be separated simultaneously in minutes.
- Potential adulteration, toxicity, and biological activity identified in seconds.
- Available in kit format.

Bioluminex Assay

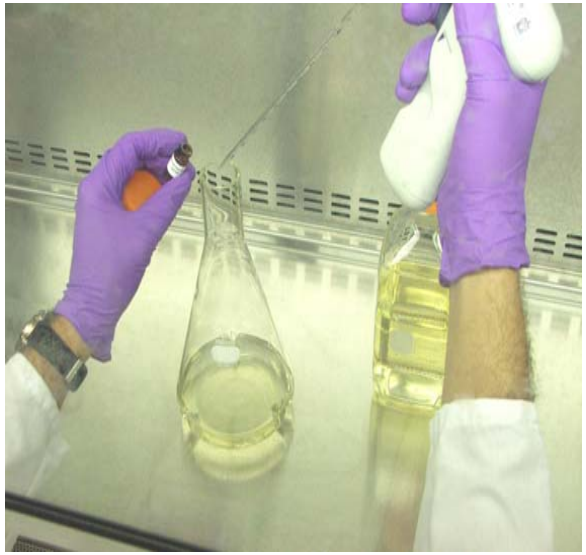
- Complex mixtures are first separated into discrete zones by TLC.
- The TLC plate is then coated with the bioluminescent bacteria, *Vibrio fischeri*.
- Toxic compounds are easily identified as dark spots on a luminescent background.
- Degree of toxicity is proportional to luminescence inhibition.



Bioluminex Assay - Kit

Vibrio fischeri

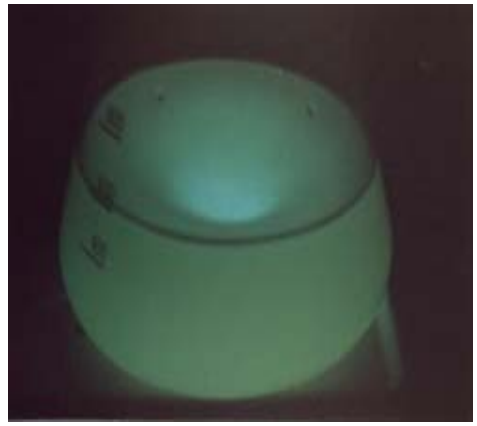
Vibrio fischeri Preparation



Bacterial Inoculation

OR

24-30 Hour Incubation



Thin Layer Chromatography

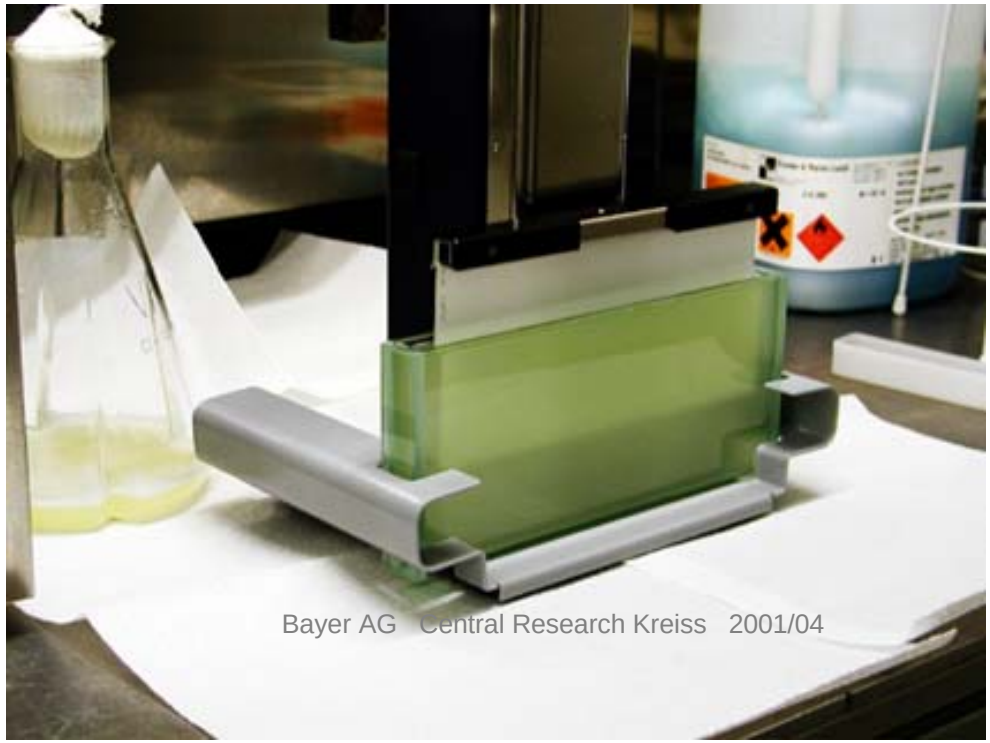
- Complex mixtures are separated by TLC using traditional TLC techniques
- Compatible with any volatile solvent including acids and bases.
- Solvent removed by evaporation.
  - Fume hood
  - Hot plate 40 °C
  - Mechanical oven 40 °C

CAMAG Automatic Developing Chambers

CAMAG Horizontal Developing Chambers

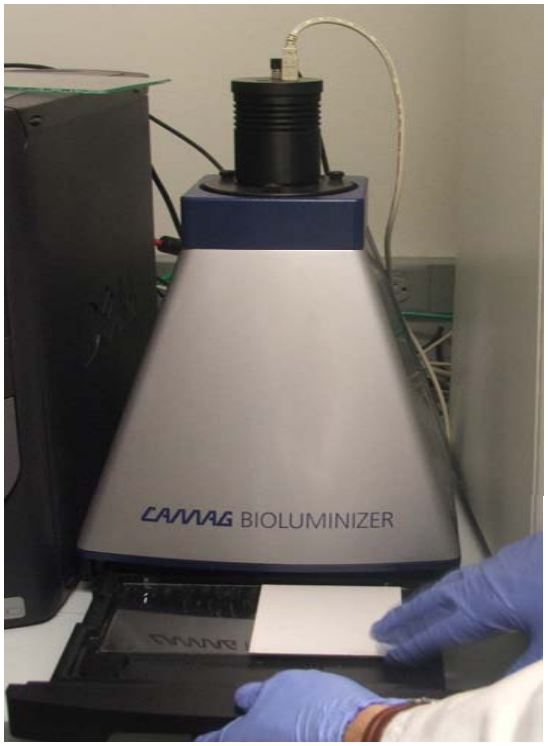
CAMAG Twin Trough/Flat Bottom Chambers

Coat TLC plate with *Vibrio fischeri*



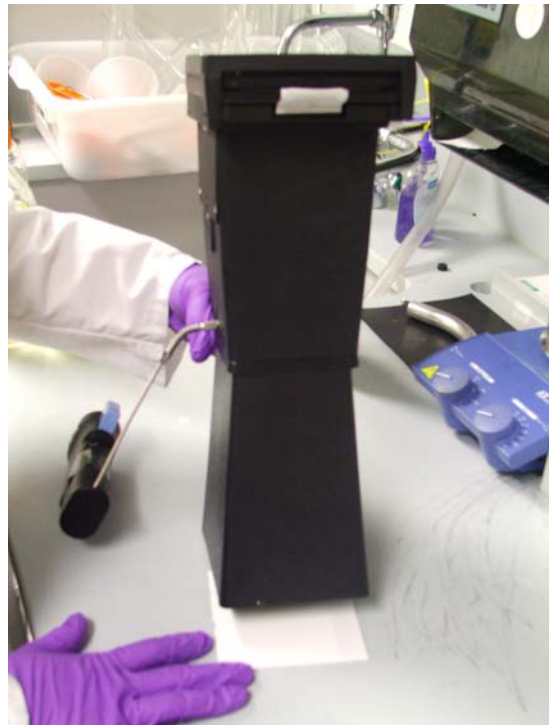
CAMAG Automatic Immersion Device

Detection Equipment



Cooled CCD Camera CAMAG BioLuminizer

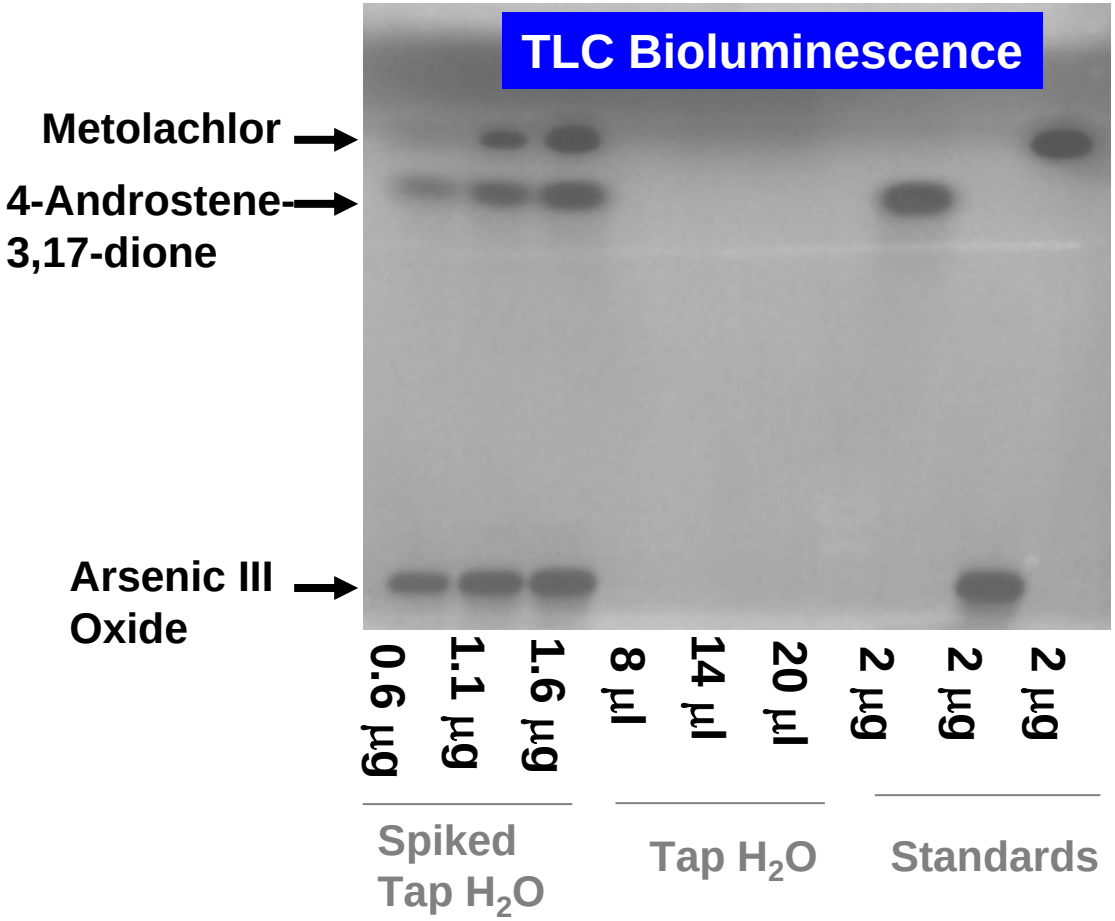
X Ray Film Processor Konica SRX 201



Polaroid-FisherBiotech Photodocmentation System w/ dark hood

Results in 1 Sec – 10 min

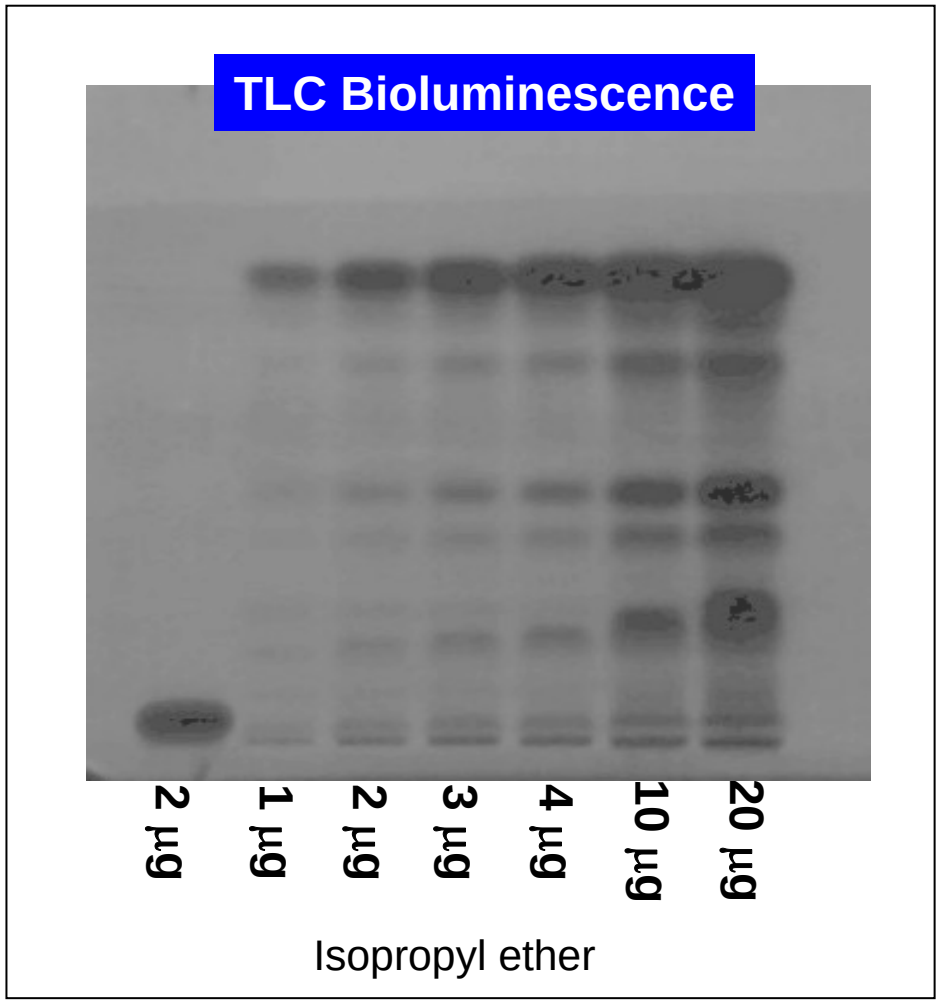
- **Decreased bioluminescence on a luminscent background indicates toxic substance zones (dark zones).**



toluene: ethyl acetate: formic acid: H_2O (4:8:1.:0.2) v/v/v

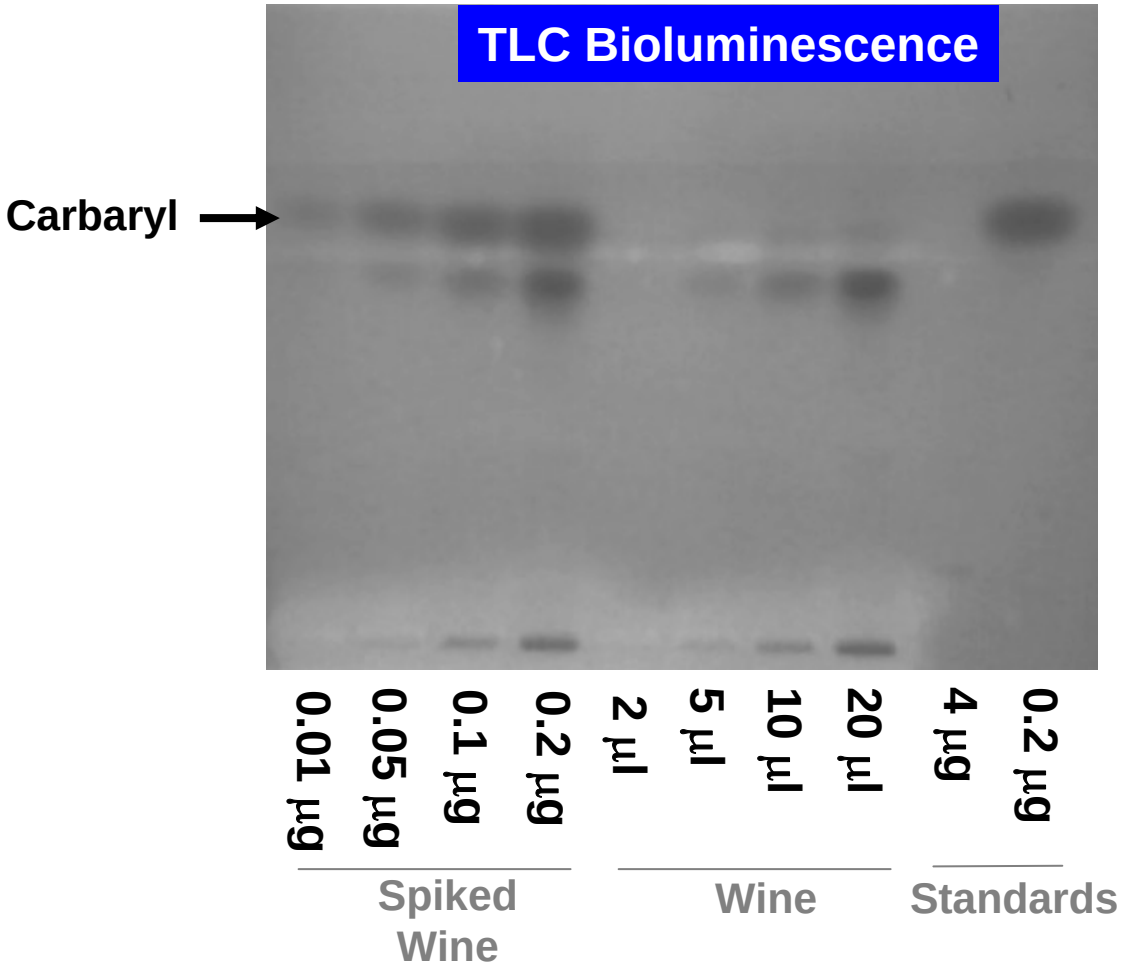
0.08 mg/ml per analyte

Fingerprint of *Capsicum annum*



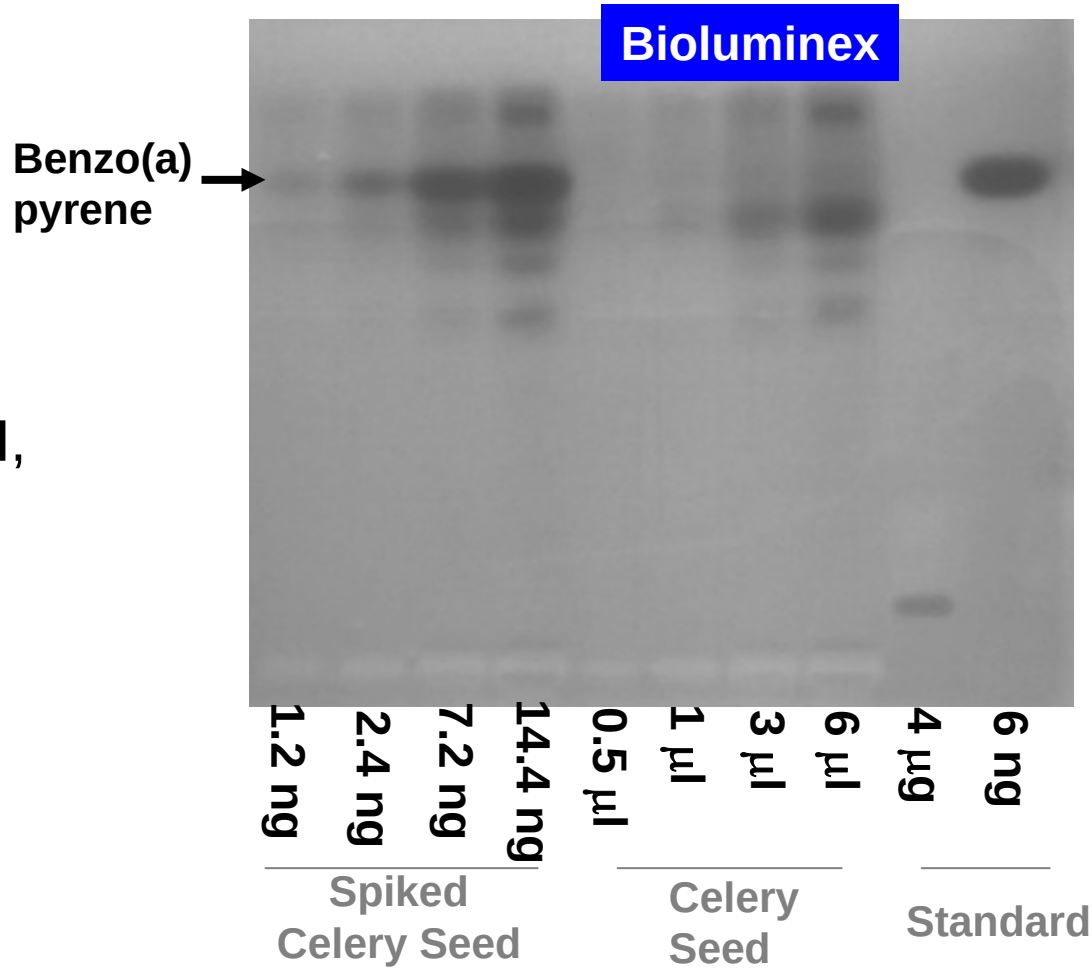
Carbaryl Spiked White Zinfandel

- White zinfandel (5 mL) was spiked with carbaryl (0.11 mg).
- Processed through a diol SPE cartridge to remove sugars.
- Developed in toluene: ethyl acetate: formic acid: H_2O (4:8:1.1:0.2) v/v/v



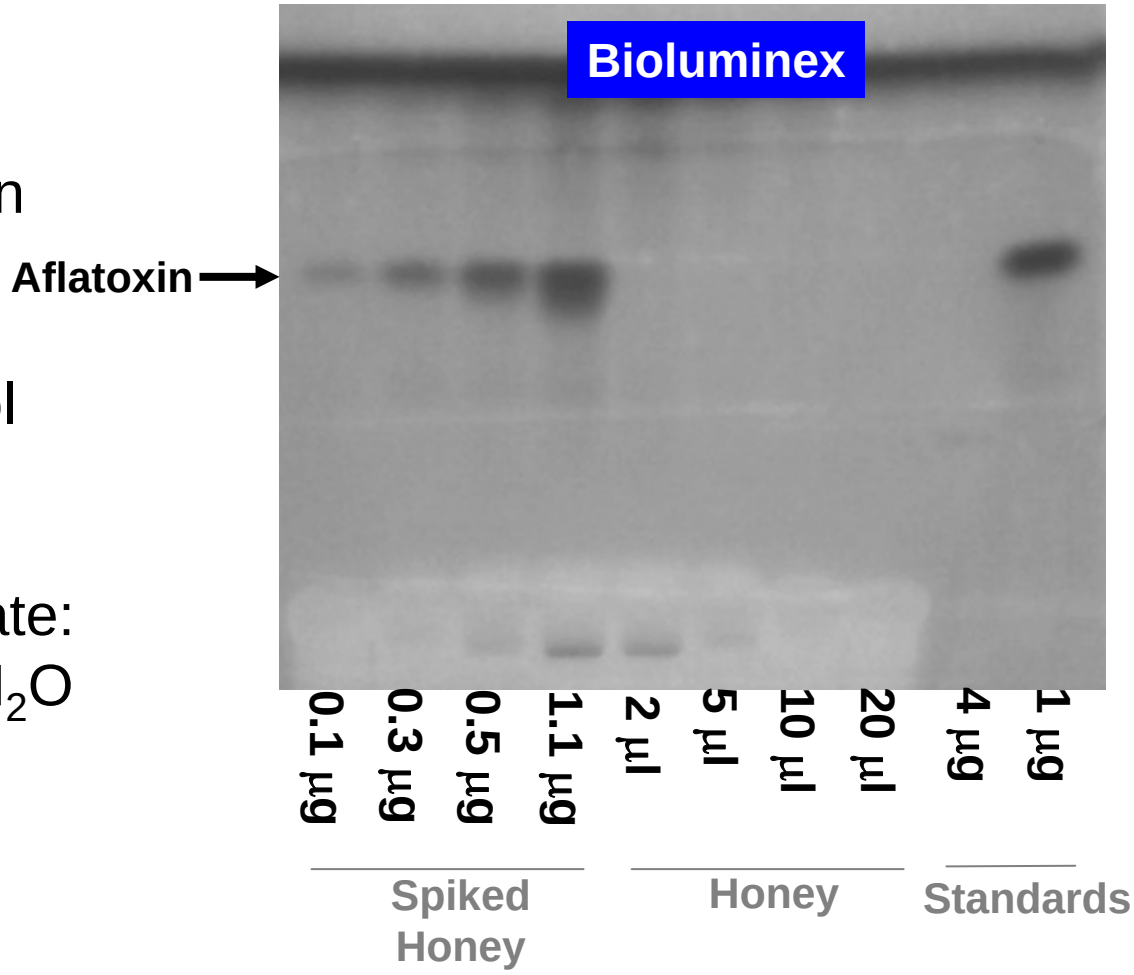
Benzo(a)pyrene Spiked Celery Seed

- Celery seed (1 g) extracted with CH_3OH (10 mL).
- Extract (1 mL) was spiked with 0.02 mg benzo(a)pyrene and diluted with 5 mL CH_3OH, 2 μg/mL.
- Developed in toluene: ethyl acetate: formic acid: H_2O (4:8:1.8:0.2) v/v/v



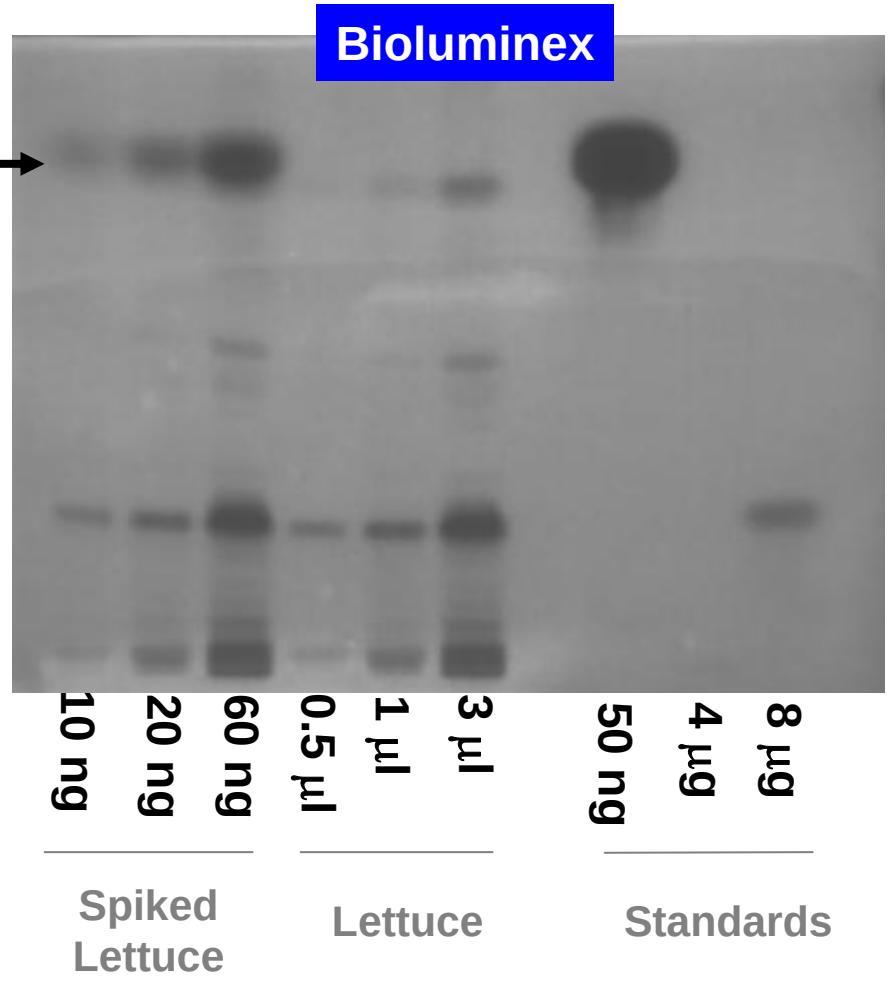
Aflatoxin B_1 Spiked Honey

- Honey (9.4 g) was spiked with Aflatoxin B_1 (0.61 mg).
- Processed through a diol SPE cartridge.
- Developed in ethyl acetate: methanol: formic acid: H_2O (50:2:5:3) v/v/v



Diphenylamine Spiked Lettuce

- Freeze-dried iceberg lettuce was homogenized and extracted (1.1 g) with CH_3OH (10 mL, 65 °C, 1 H).
- Filtered lettuce extract (2 mL) was spiked with diphenylamine (40 μg, 20 μg/mL)
- Developed in toluene: ethyl acetate: formic acid: H_2O (7:5:1.1:0.2) v/v/v



THANK YOU

For more information on Bioluminex™ please visit www.bioluminex.com To order the Bioluminex™ kit please use the following contact information.

It's easy to order from ChromaDex™

1. Call: 949.419.0288
   Monday to Friday
   6:00am - 5:30pm PST
2. Fax: 949.419.0294
3. Web: www.chromadex.com
4. Mail: 2952 S. Daimler St.
   Santa Ana CA 92705

Shipping: (USA, Canada and International)


USA: Fed Ex, UPS, DHL


Canada: Fed Ex, UPS, DHL


International: Fed Ex, UPS, DHL

*Other freight companies are available on request.

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Protected by patents: EP 0588 139 B1 & US 6,238,928 & JP 0 246 032-93 & 6,673,563 & 6,340,572 & 6,017,722