

HPTLC-bioassay-MS, a rapid tool to search and analyse bioactive plant products

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There is a demand for new effective agents applicable in human and animal medicine as well as in plant protection.



Alexander Flemming

The origin of the vast number of diseases is infection by microorganism.

The increasing incidence of the resistance of pathogens against widely used antibiotics means a big issue in the medicine.

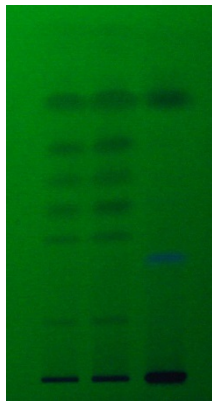


Direct bioautography (DB)

search of matrix components having antimicrobial effect

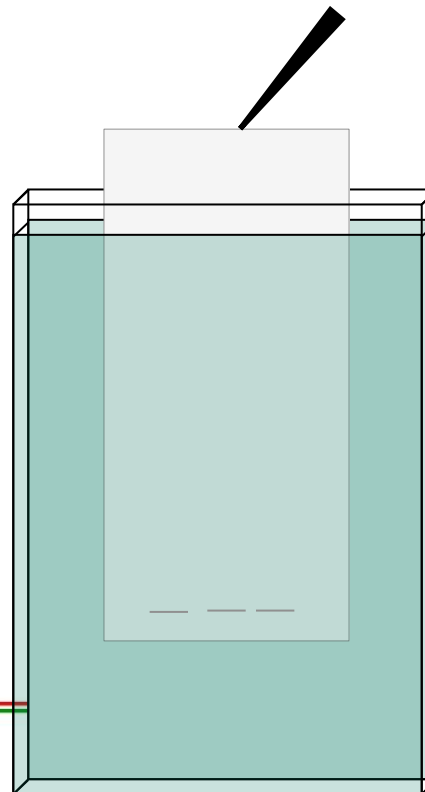
fast, simple, relatively cheap, high-throughput investigation

separation in thin layer
(e.g. TLC, HPTLC, OPLC)

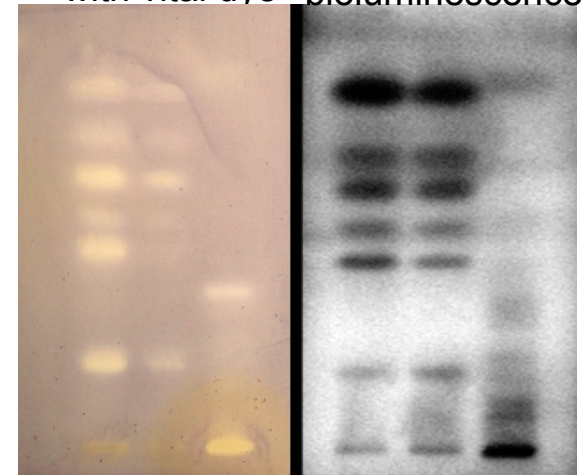


→ „inoculation” ^{incubation} → visualisation

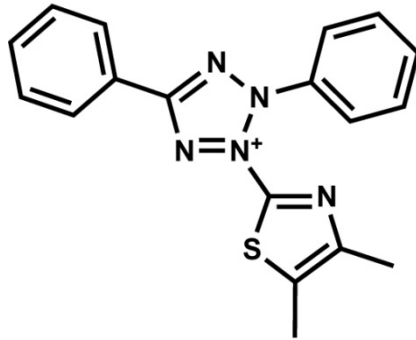
The developed adsorbent layer is dipped into the cell suspension



with vital dye bioluminescence

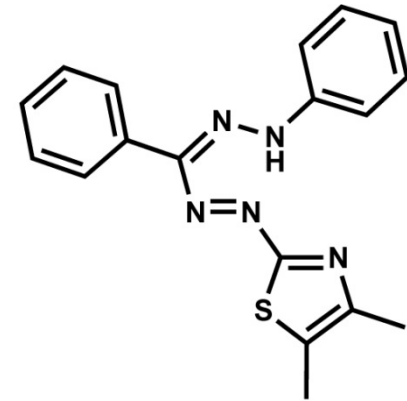


with vital dye



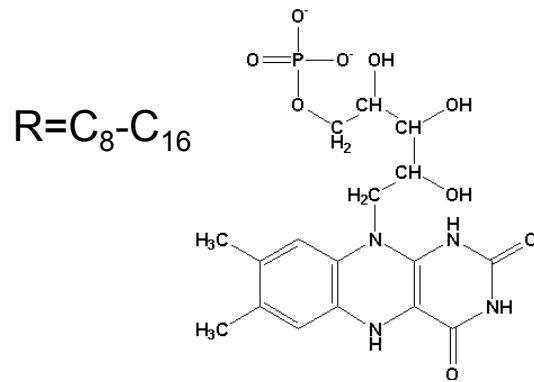
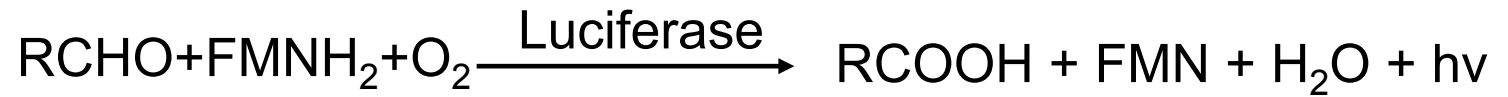
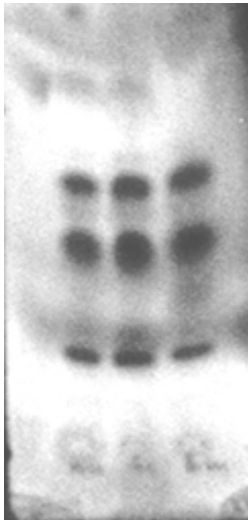
MTT - yellow tetrazolium

mitochondrial
reductase
enzymes



formazan dye - purple

bioluminescence



FMNH₂

otid



Characterization of biologically active components by techniques linked to layer chromatography

In situ

Densitometry - obtaining spectra

IR, FT-IR, Raman and FT-Raman spectroscopy

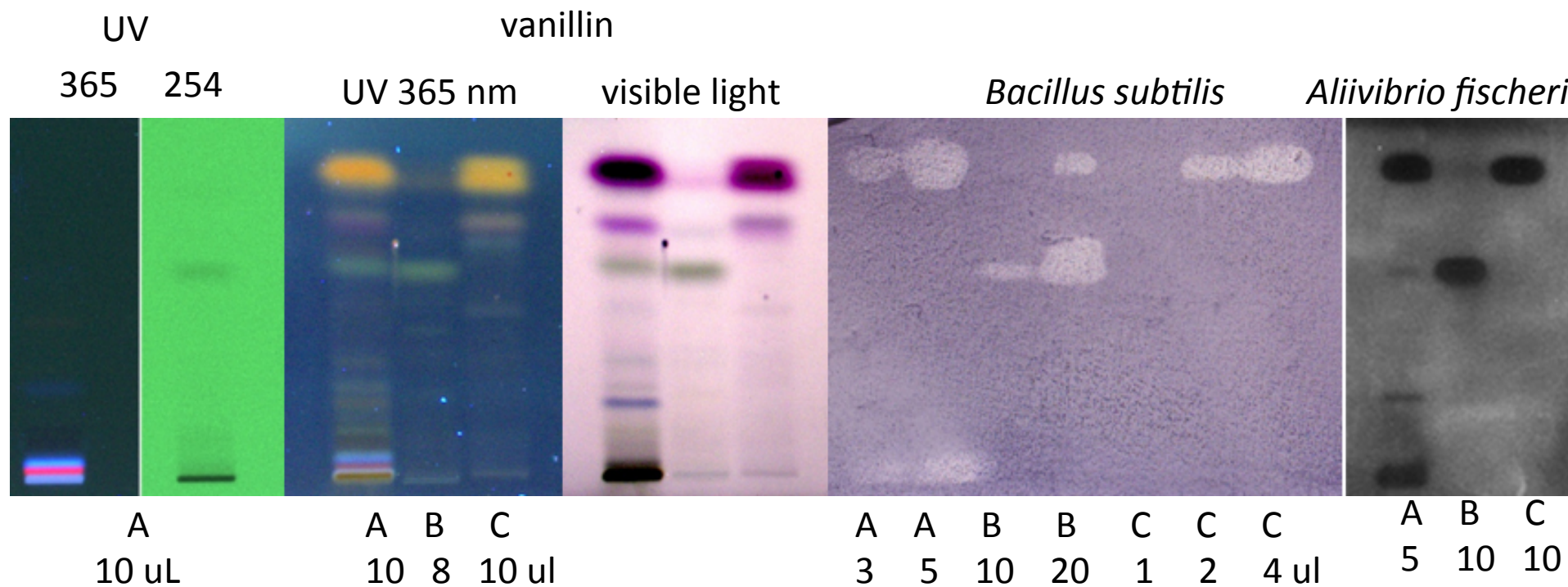
MS [e.g. DART (thermic desorption), DESI (charged liquid or gas stream), MALDI (laser), laserablation inductive coupled plasma MS (LA-ICP-MS)]

Ex situ

The analysis of the eluted compounds - what you can imagine
(TXRF, MS, GC-MS, LC-MS or MS/MS, NMR, etc.)



1st example

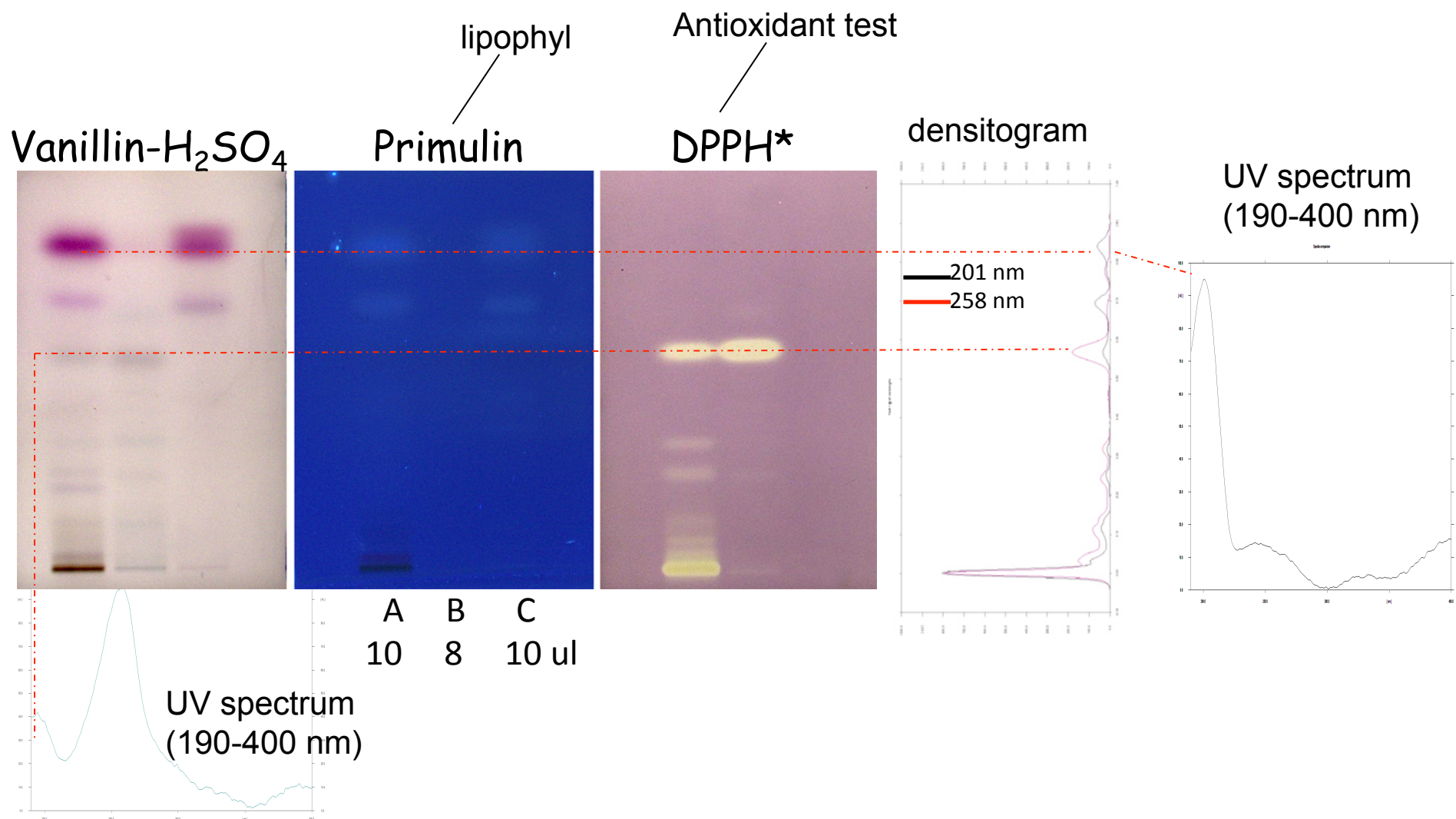


A - crude extract
 B, C - OPLC fractions

HPTLC Silica gel 60 (without F)
 19% isopropyl acetate and 1% acetic acid in hexane



Application of reagents

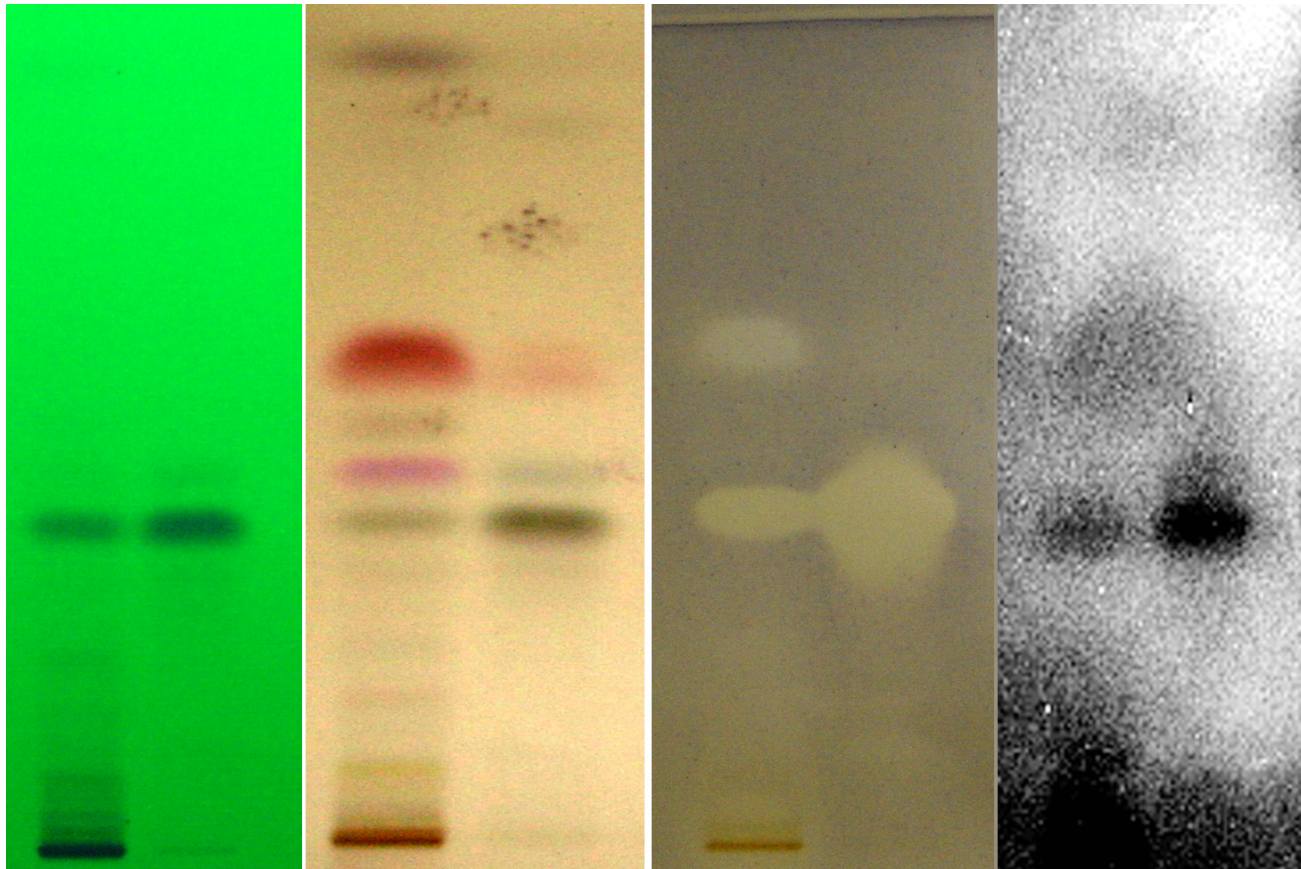


UV 254

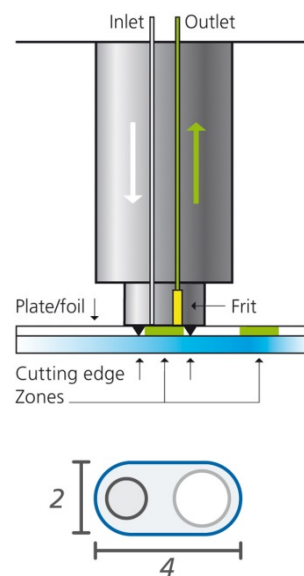
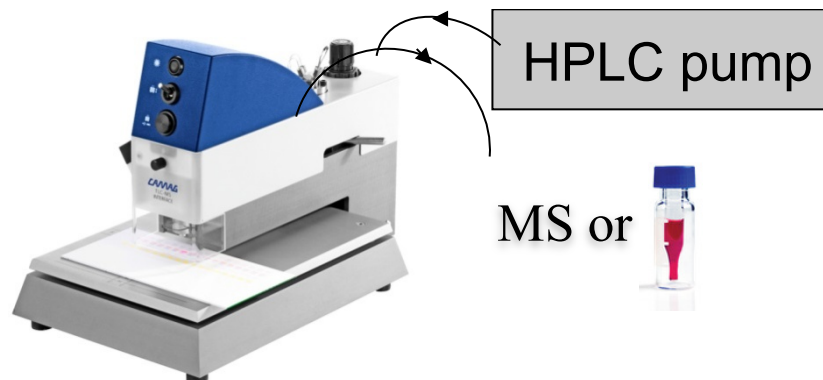
Vanillin

X. euvesicatoria

P. maculicola

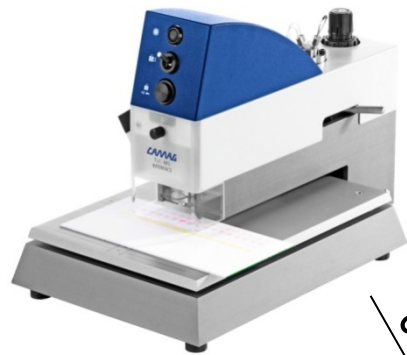


Elution by TLC-MS-Interface



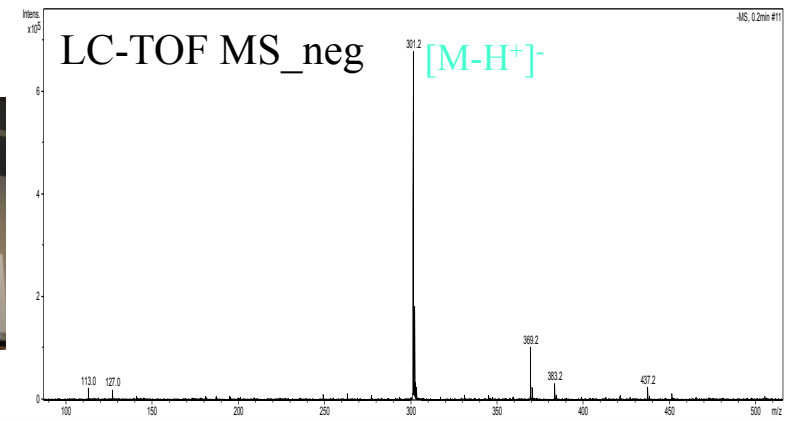
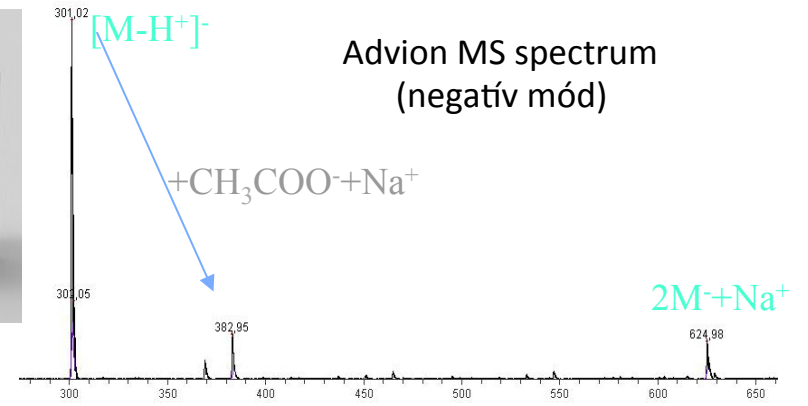


elution
with
TLC-MS-Interface



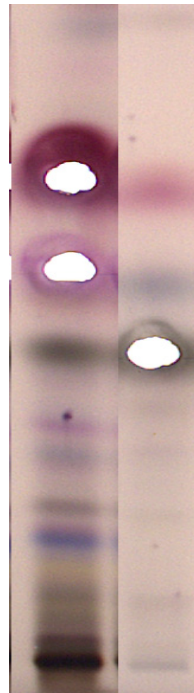
on-line

off-line

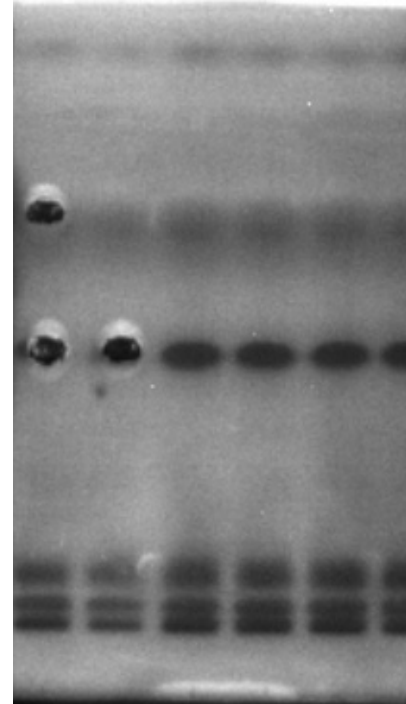


Was the position perfect?

vanillin

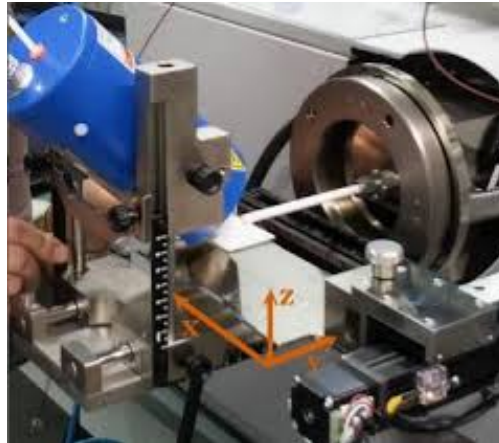


Aliivibrio fischeri



DART SVP-A-MS (Direct Analysis in Real Time)

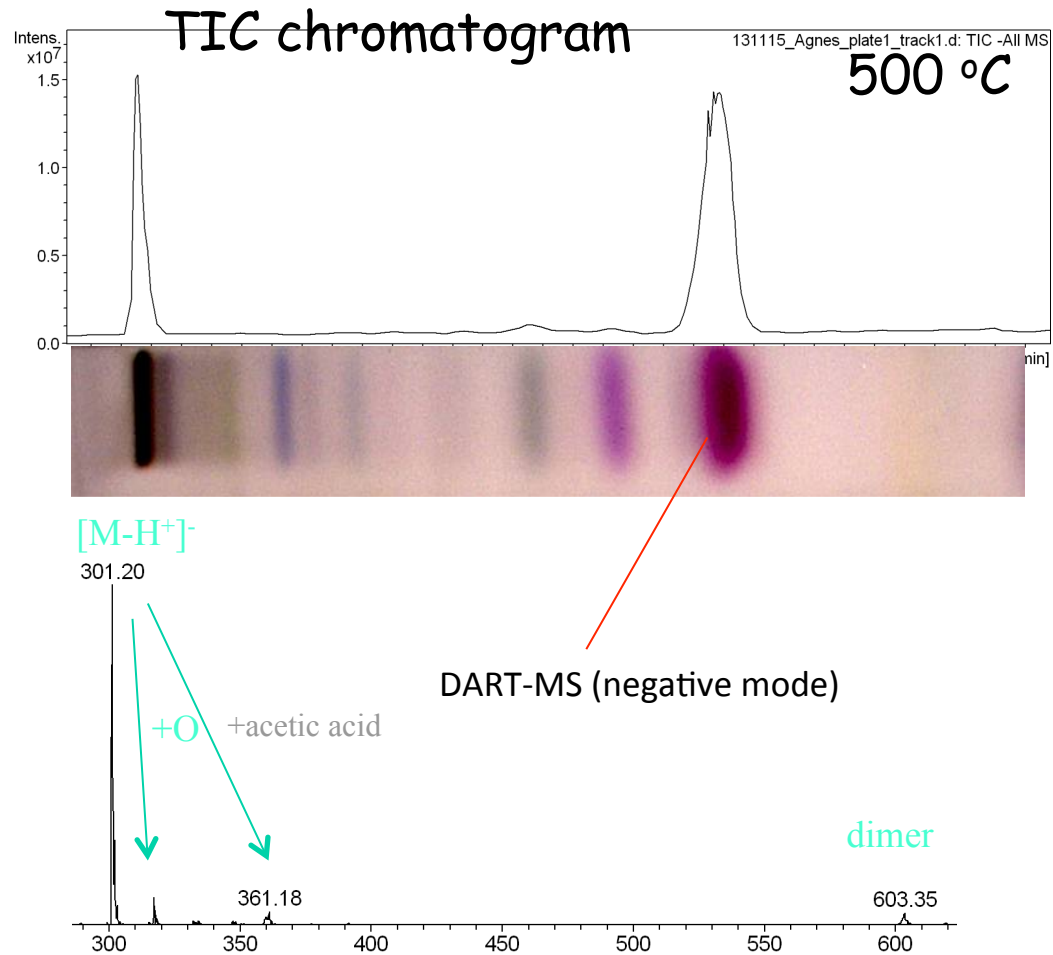
Limitation – we must somehow transport the analyte from the adsorbent layer into the MS by warm gas stream



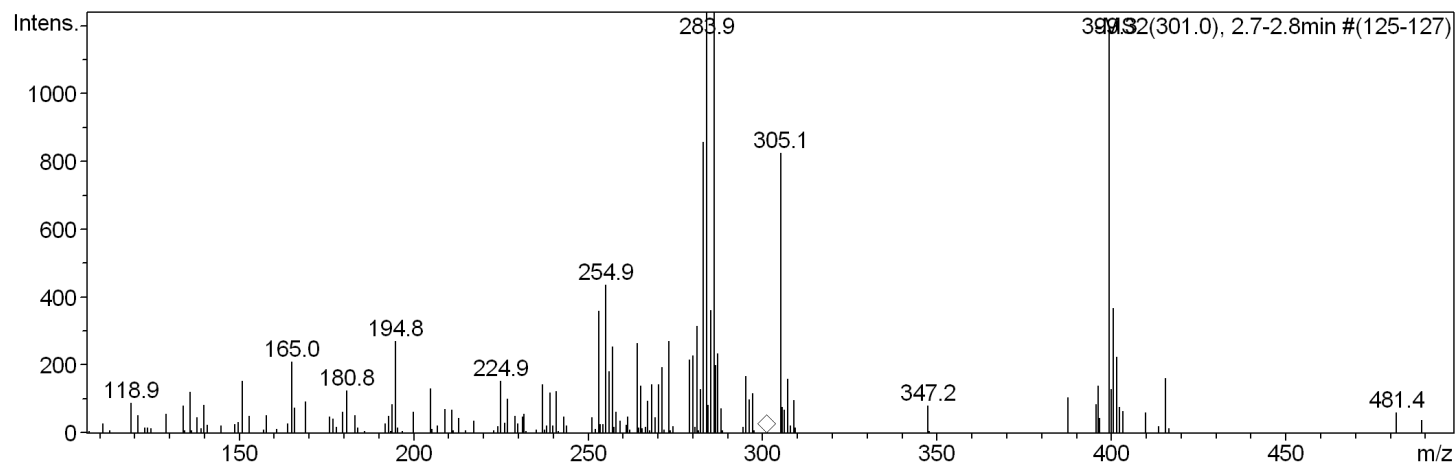
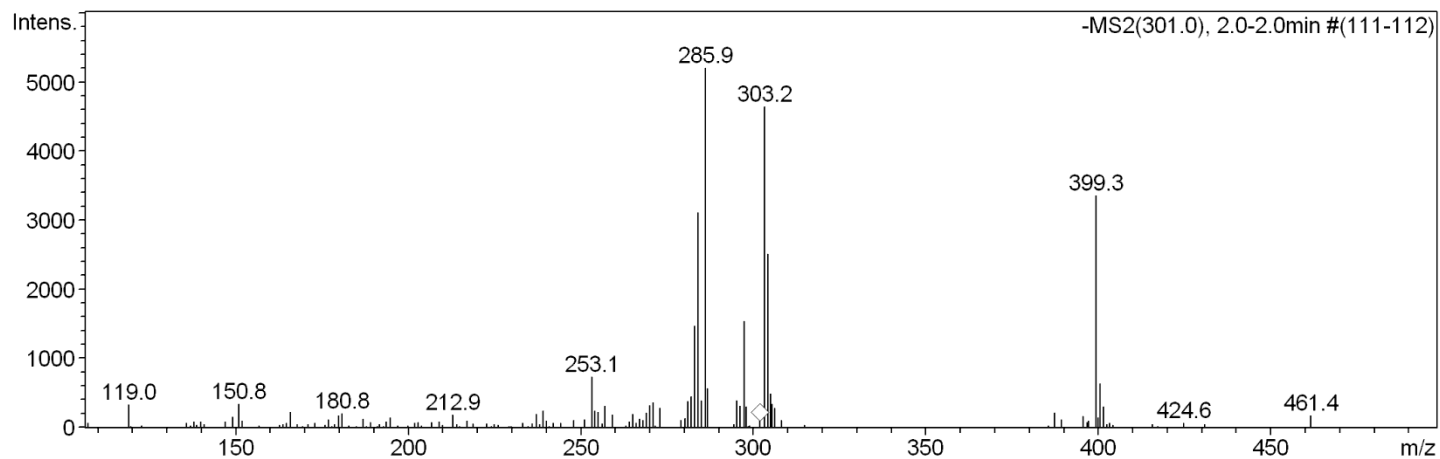
Stream of heated, excited gas without charge



DART-MS (Direct Analysis in Real Time)



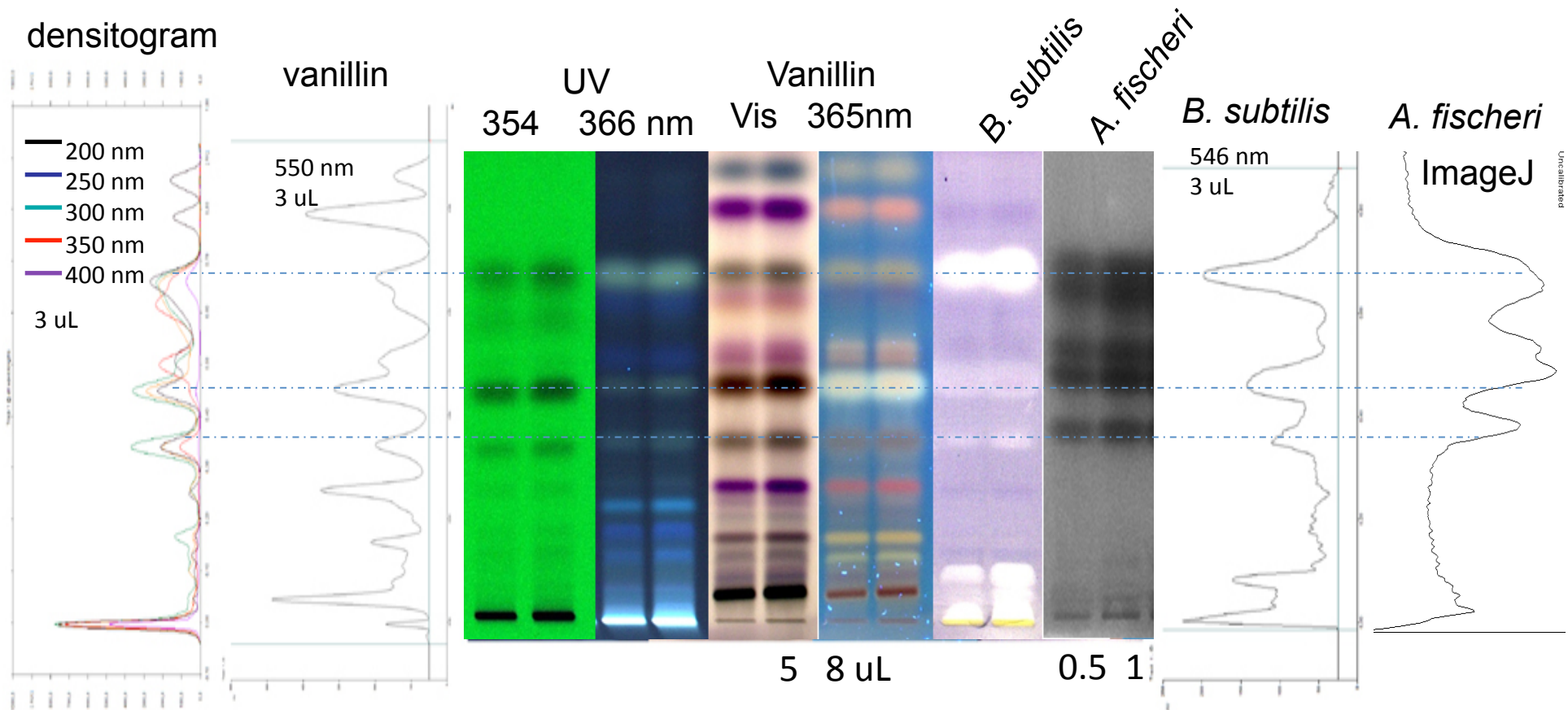
HPTLC-DART-MS/MS



Higher E



2nd example

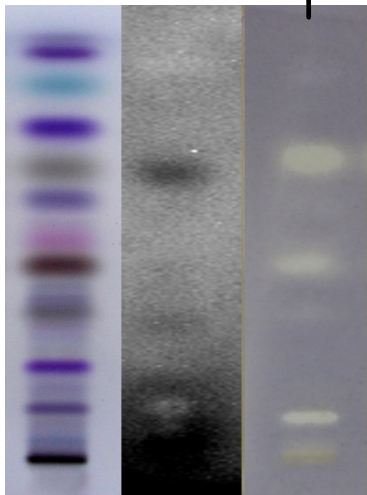


HPTLC Silica gel 60 (without F)
10% isopropyl acetate in hexane

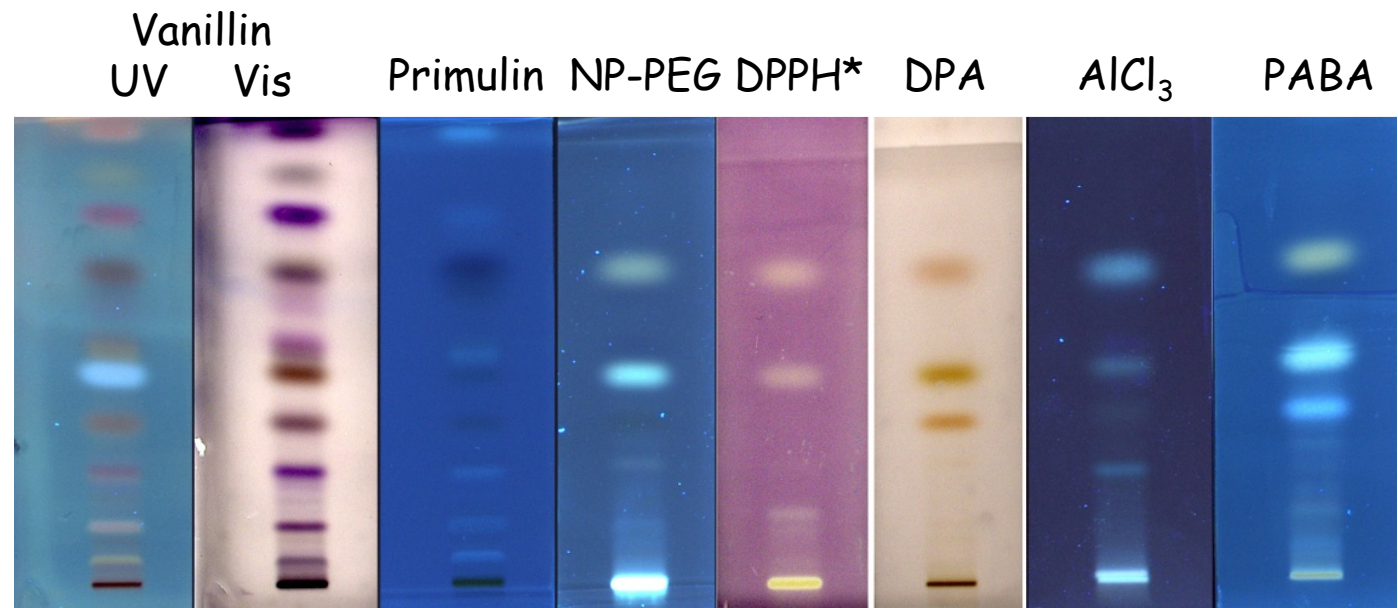


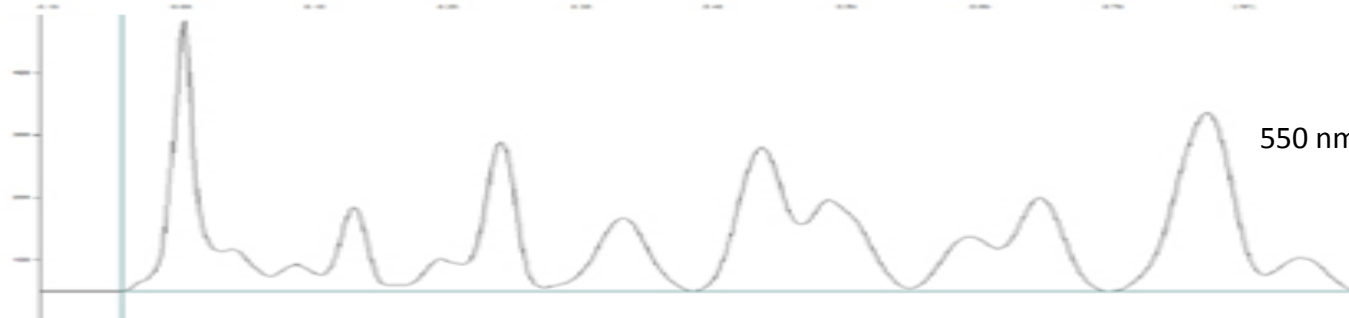
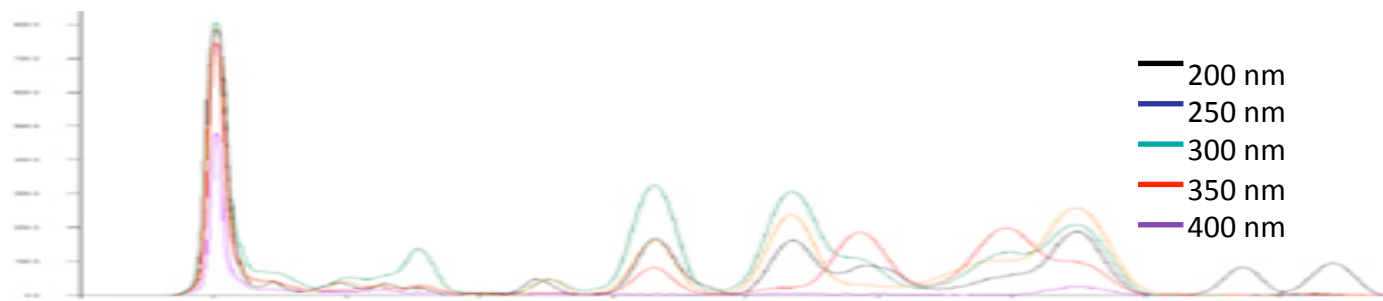
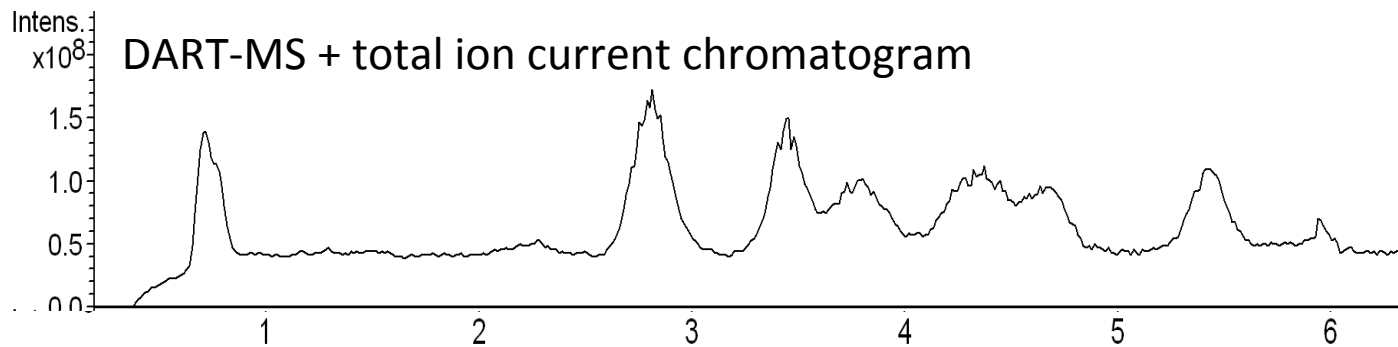
Testing against other bacteria strains

Vanillin
P. maculicola
X. euvesicatoria



The use of various reagents





vanillin

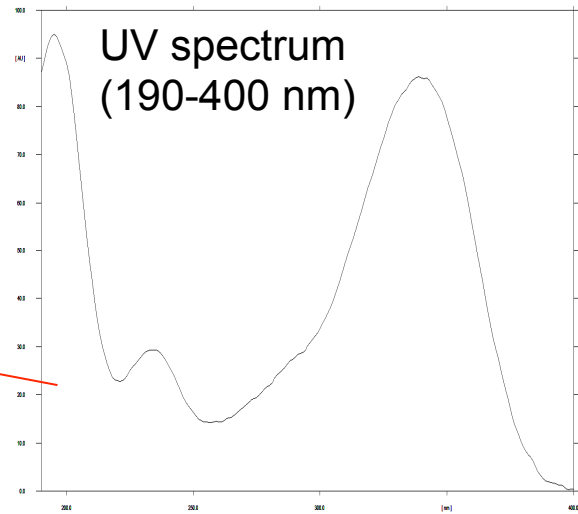


vanillin

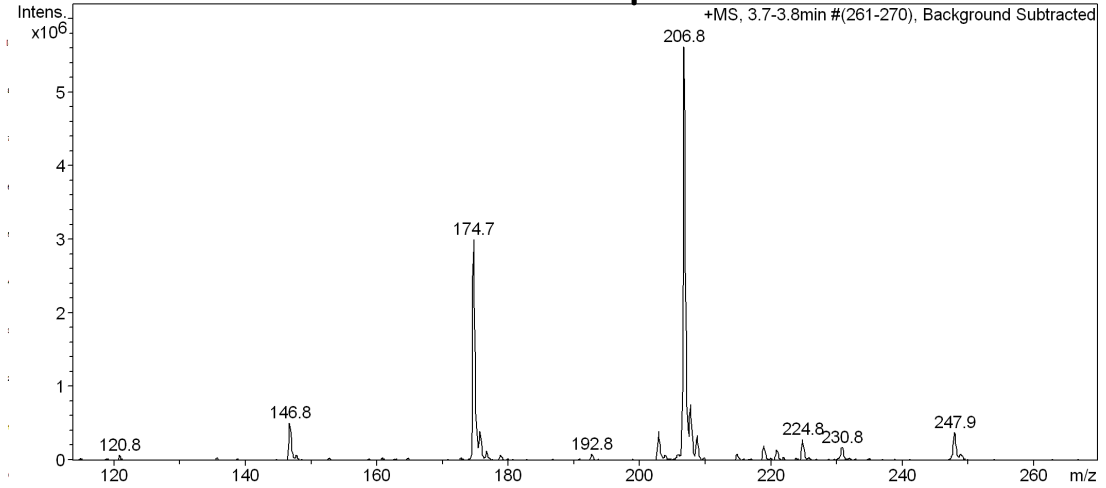
Antibacterial components



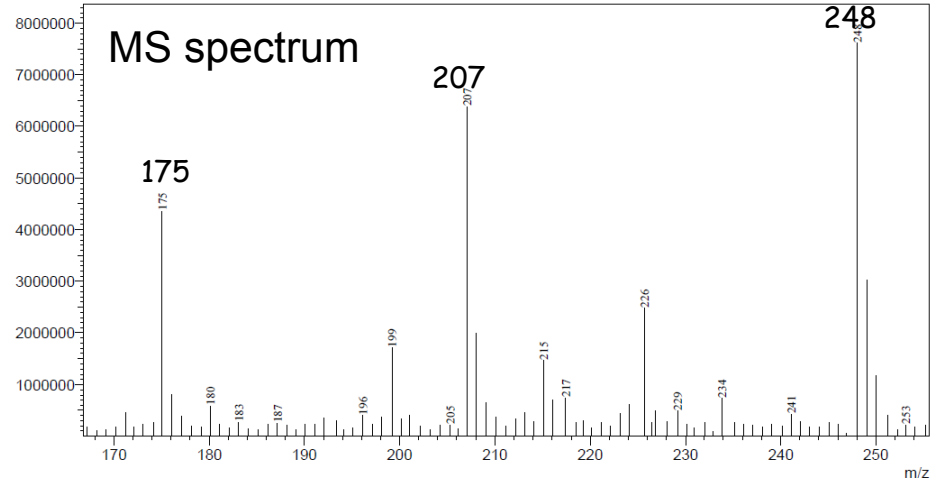
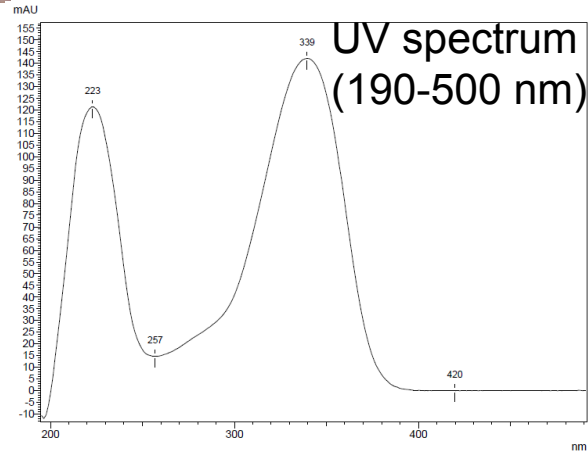
densitometer



DART-MS spectrum



LC-DAD-MS



Conclusions

The reliable high-throughput HPTLC-Bioassay-MS systems enable the search for and the characterization of the bioactive compounds from different matrices.

These systems ensure a cheaper and less time-consuming way for the isolation of substances with desired biological activity.



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Thank you for your kind attention!

