

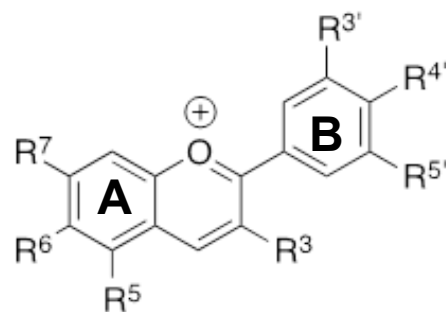
A rapid identification of anthocyanins in various plants from Burkina Faso by HPTLC/MS



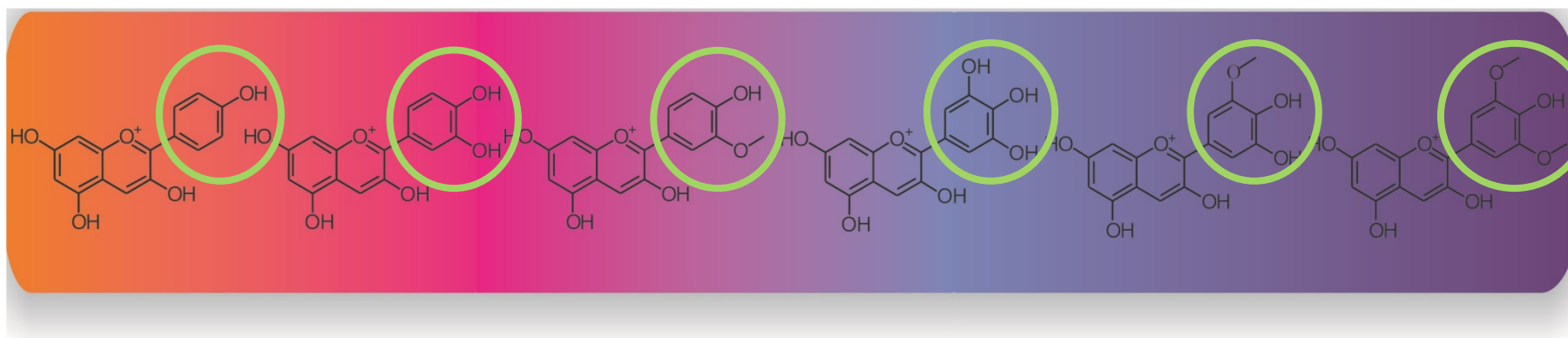
Presented by
Rosella SPINA



Background- Anthocyanidins chemistry



Anthocyanidins
R-groups: -H, -OH and / or -OCH₃



Pelargonidin

Cyanidin

Peonidin

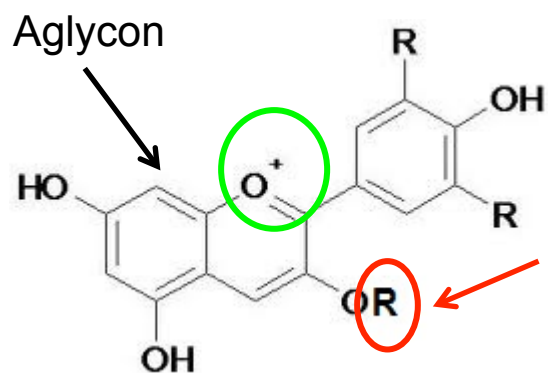
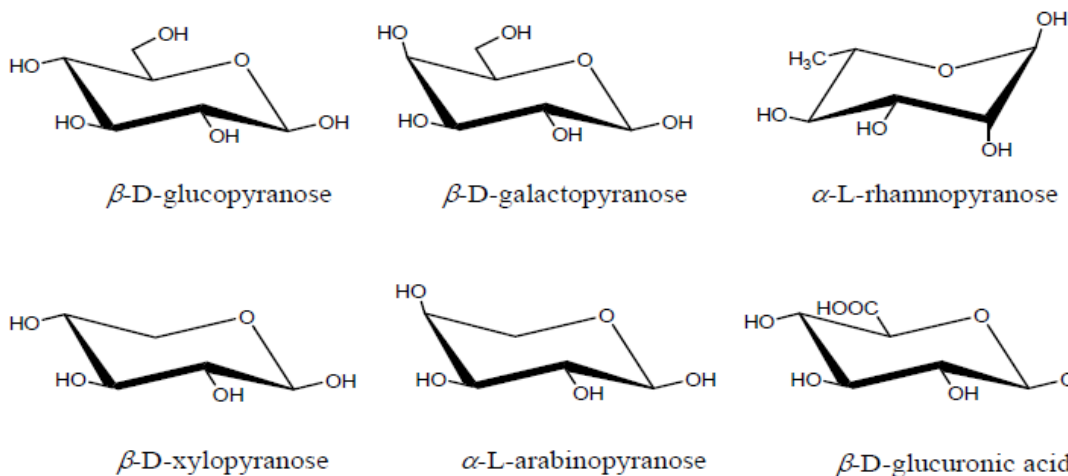
Delphinidin

Petunidin

Malvidin

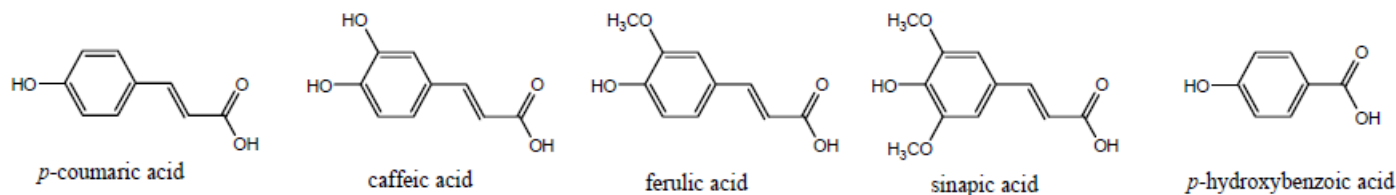
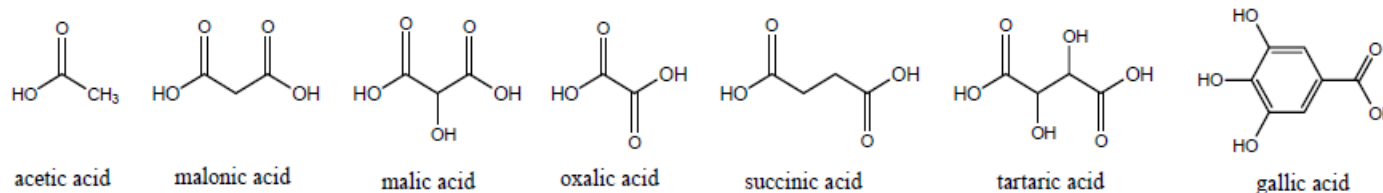
> 300 structurally different anthocyanins

Sugars



Acyl moiety

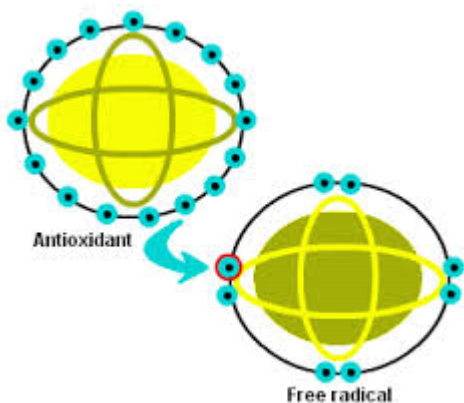
2-Phenylbenzopyrylium cation



Are used as a food-colorant

E163

Have antioxidant properties



Potential therapeutic role to:

- Cardiovascular diseases
- Cancer
- Inhibition of several virus
- Improvement of visual acuity

Burkina Faso's plants studied

HPTLC separation conditions

for qualitative and quantitative analysis of anthocyanins



Tapinanthus dodoneifolius
Loranthaceae
Flowers

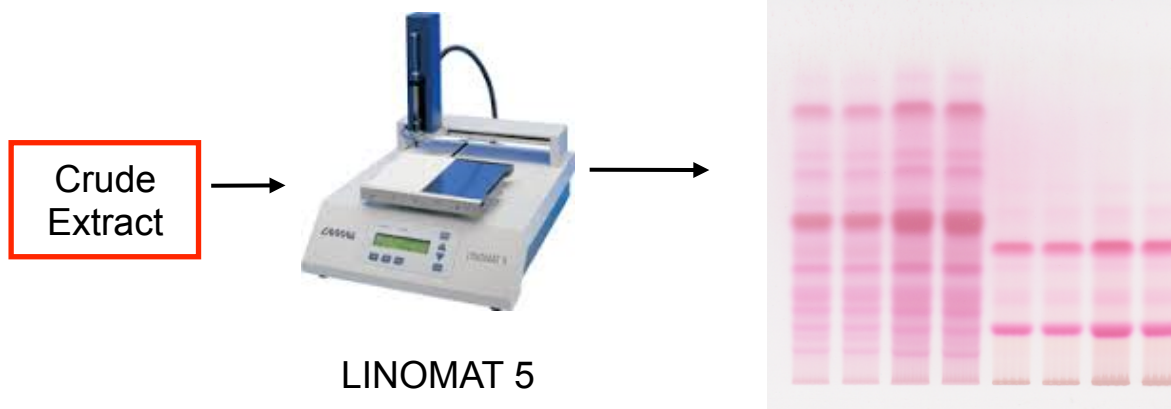


Burkina Faso



Eichhornia crassipes
Pontederiaceae
Flowers

Screening stationary and mobil phase



HPTLC plate silica gel 60 F₂₅₄

Different solvents systems:

-) *n*-Butanol-acetic acid-water 40-10-20 v/v
-) Ethyl acetate-isopropanol-water 65-25-10 v/v
-) Ethyl acetate-toluene-water-formic acid 10-3-0.8-1.1 v/v
-) Ethyl acetate-butanone-water-formic acid 7-3-0.8-1.2 v/v
-) Ethyl acetate-acetic acid-formic acid, water 110-11-11-25 v/v

HPTLC plates RP-18 WF_{254S}

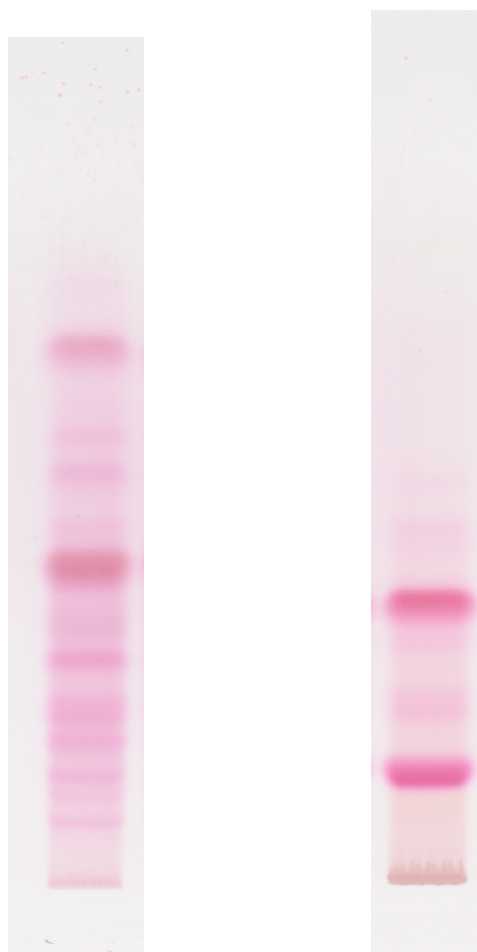
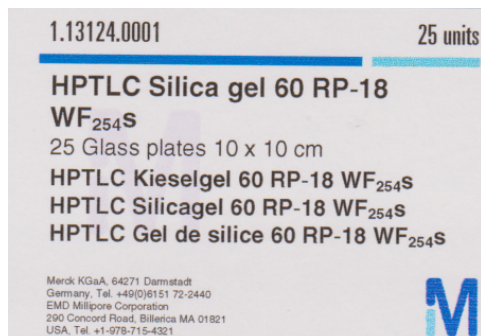
Different solvents systems:

-) Methanol-water- formic acid 40-60-2 v/v
-) Methanol-water-TFA 55-45-1 v/v
-) Methanol-water-TFA 40-60-5 v/v
-) Ethanol-water-TFA 20/80/2 v/v
-) Acetonitrile-water-TFA 20-80-2 v/v

Optimized conditions of separation



LINOMAT 5



*Eichhornia
crassipes*

*Tapinanthus
dodoneifolius*

HPTLC plates
RP-18 WF_{254S}

Acetonitrile-water-TFA
20-80-2 (v/v)

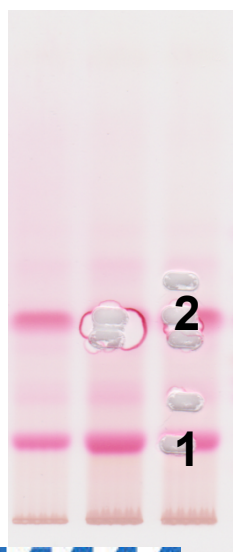
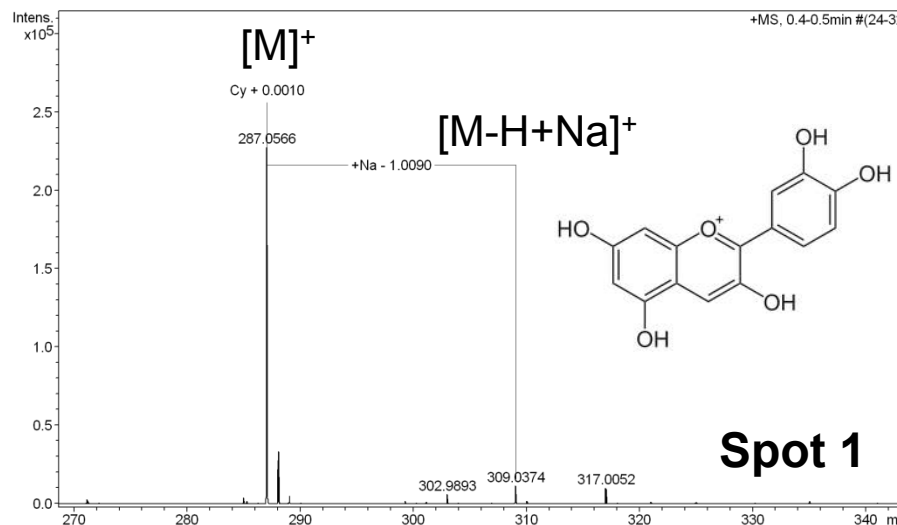
Results by HPTLC/MS from crude extract of *Tapinanthus dodoneifolius*



Tapinanthus dodoneifolius
Loranthaceae
Flowers



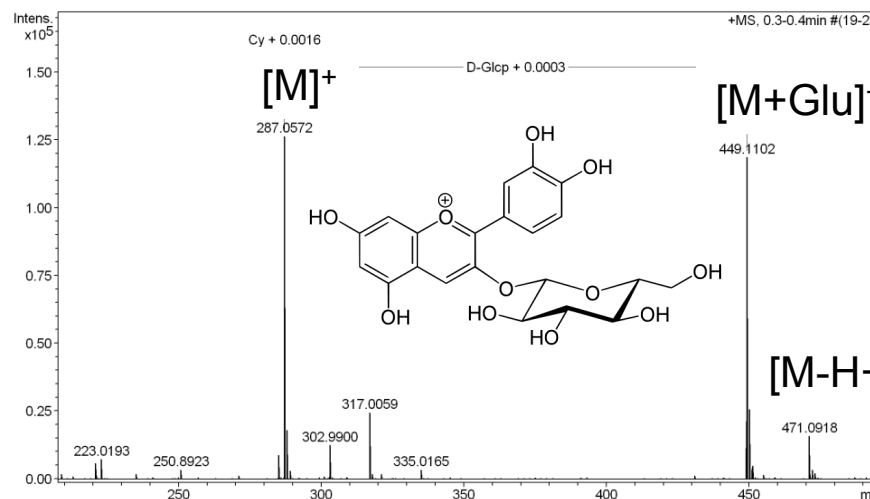
MicroTofQ
Bruker



CAMMAG



TLC/MS interface



Spot 2



Results by HPTLC/MS from crude extract of *Eichhornia crassipes*



Eichhornia crassipes
Pontederiaceae
Flowers



		[M] ⁺	MS/MS
Spot 11	No detectable	-	-
Spot 10	Delphinidin-3,5-diglucoside	627.1586	465.1057/ 303.0414
Spot 9	Delphinidin diglucoside + malic acid	713.1622	551.1121/465.1029/ 303.0514
Spot 8	Delphinidin diglucoside +succinic acid	727.1793	565.1376/ 303.0514
Spot 7	Delphinidin derivatives 2	611.1648	303.0414
Spot 6	Delphinidin diglucoside +succinic acid	727.1736	565.1172/465.1032/ 303.0492
Spot 5	Delphinin- derivatives 1	551.1075	303.0509
Spot 4	Delphinidin-3-glucoside	465.1062	303.0509
Spot 3	Pelargonidin-3-glucoside	433.1016	271.0530
Spot 2	Pelargonidin 3-(6' ' coumaroyl) glucoside	579.1379	271.0530
Spot 2	Delphinidin	303.0483	
Spot 1	Cyanidin	287.0470	



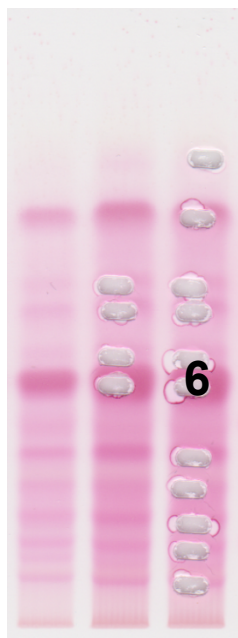
Results from HRMS



Example of HPTLC/MS from *Eichhornia crassipes*



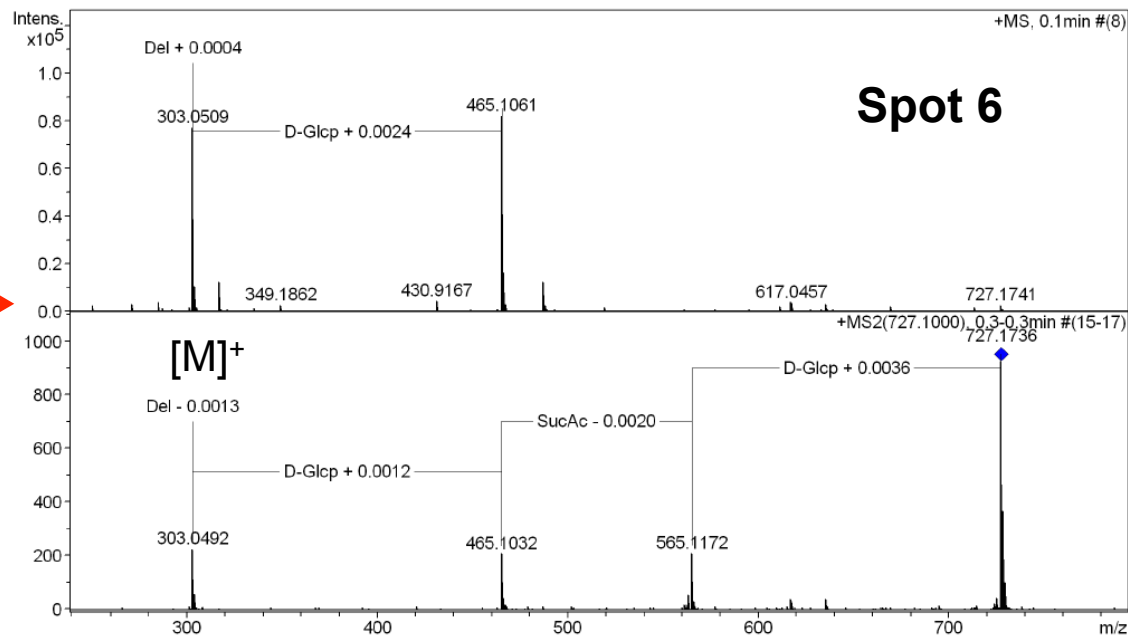
Eichhornia crassipes
Pontederiaceae
Flowers



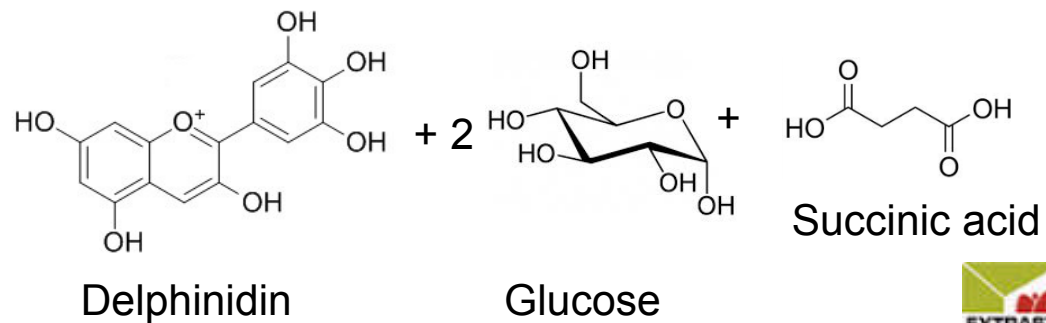
MicroTofQ
Bruker



TLC/MS interface



MS and MS/MS spectra





**SCANNER TCL 4
CAMAG**



Sample	Compound	nm	ng/spot	r	sdv %
<i>Tapinanthus dodoneifolius</i>	Cyanidin	530	1918	0.99782	2.06
<i>Eichhornia crassipes</i>	Cyanidin	530	736.25	0.99782	2.06
<i>Tapinanthus dodoneifolius</i>	Cyanidin-3-glucoside	530	1896	0.99741	2.94
<i>Eichhornia crassipes</i>	Pelargonidin	510	605.25	0.99739	1.33
<i>Eichhornia crassipes</i>	Pelargonidin-3-glucoside	520	840.87	0.99853	1.57
<i>Eichhornia crassipes</i>	Delphinidin	555	1109	0.99292	6.33
<i>Eichhornia crassipes</i>	Delphinidin-3-glucoside	555	1712	0.99800	3.79
<i>Eichhornia crassipes</i>	Delphinidin-3,5-diglucoside	530	1816	0.99917	1.08

Correlation coefficient > 0.99

Regression via height: polynomial;

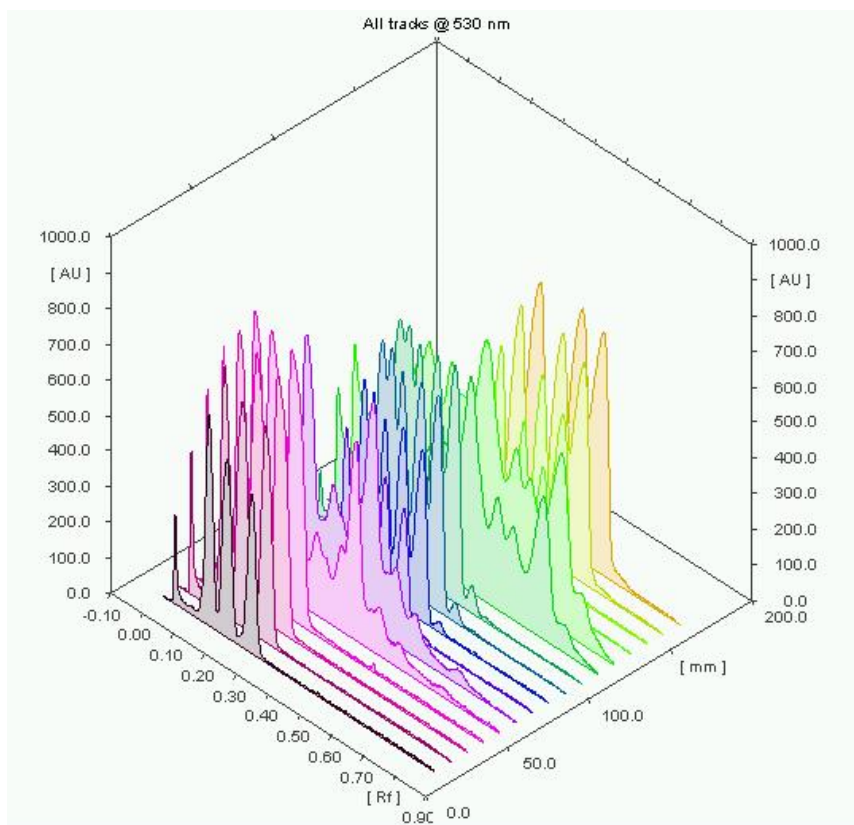
Volume applied of samples 10 μ L;

Concentration of solutions of crude extract: 5 mg/0,5 mL in MeOH

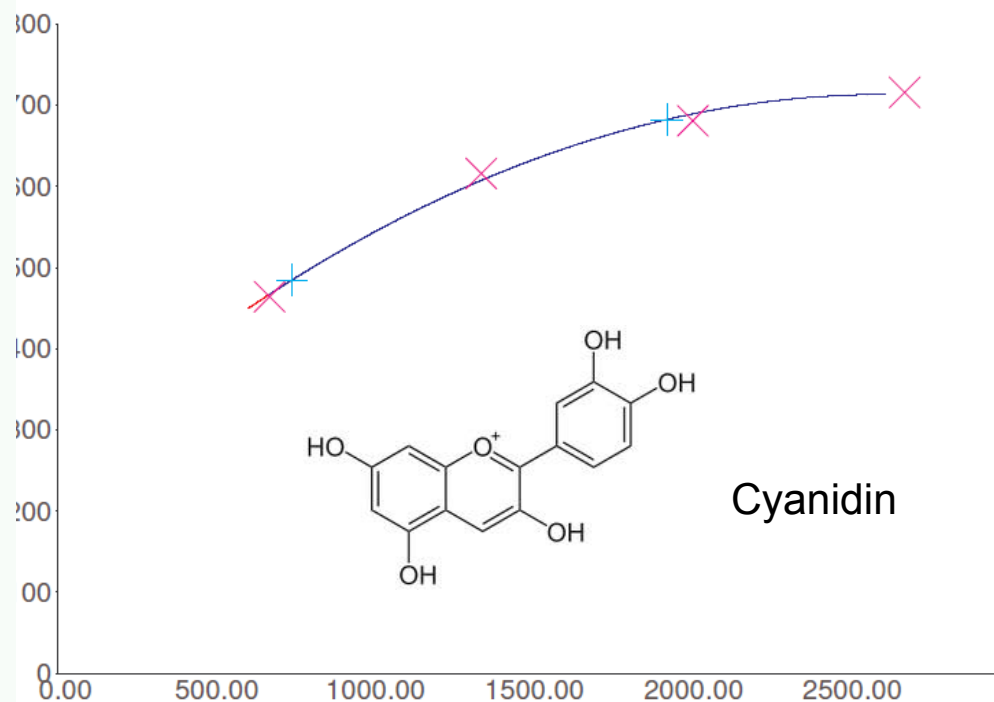
Substance: cyan @ 530 nm

Regression via height: Polynomial $Y = 268.3 + 0.3419 * X + -6.569e-005 * X^2$

$r = 0.99782$ $sdv = 2.06$



HPTLC 3D-densitogram of absorbance measurement at 530nm for all tracks



Polynomial calibration of cyanidin via peak height by absorption at 530 nm



Conclusion



Methanolic extract



Methanolic extract



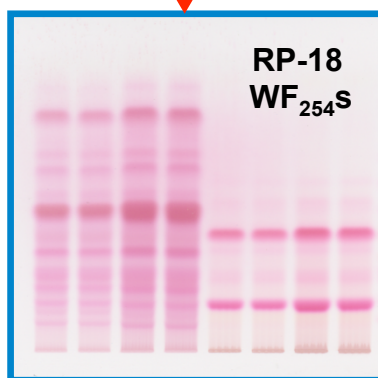
Linomat 5 CAMAG



TLC-MS interface CAMAG



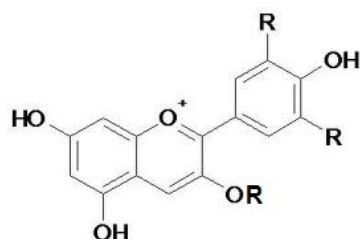
Scanner TLC 4 CAMAG



MicroTofQ BRUKER

Quantification of 7 anthocyanins

Determination of 14 anthocyanins





Pr. Dominique LAURAIN-MATTAR

Service Commun de Spectrométrie de Masse:
François DUPIRE
Dr. Fabien LACHAUD

Université Ouagadougou
Burkina faso

Dr. Adama HÉMA
Dr. Eloi PALÉ
Pr. Mouhoussine NACRO

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THANKS FOR YOUR ATTENTION

