



**HPTLC, support tool for botanical
identity testing and product
development**

FROM NATURE TO YOU

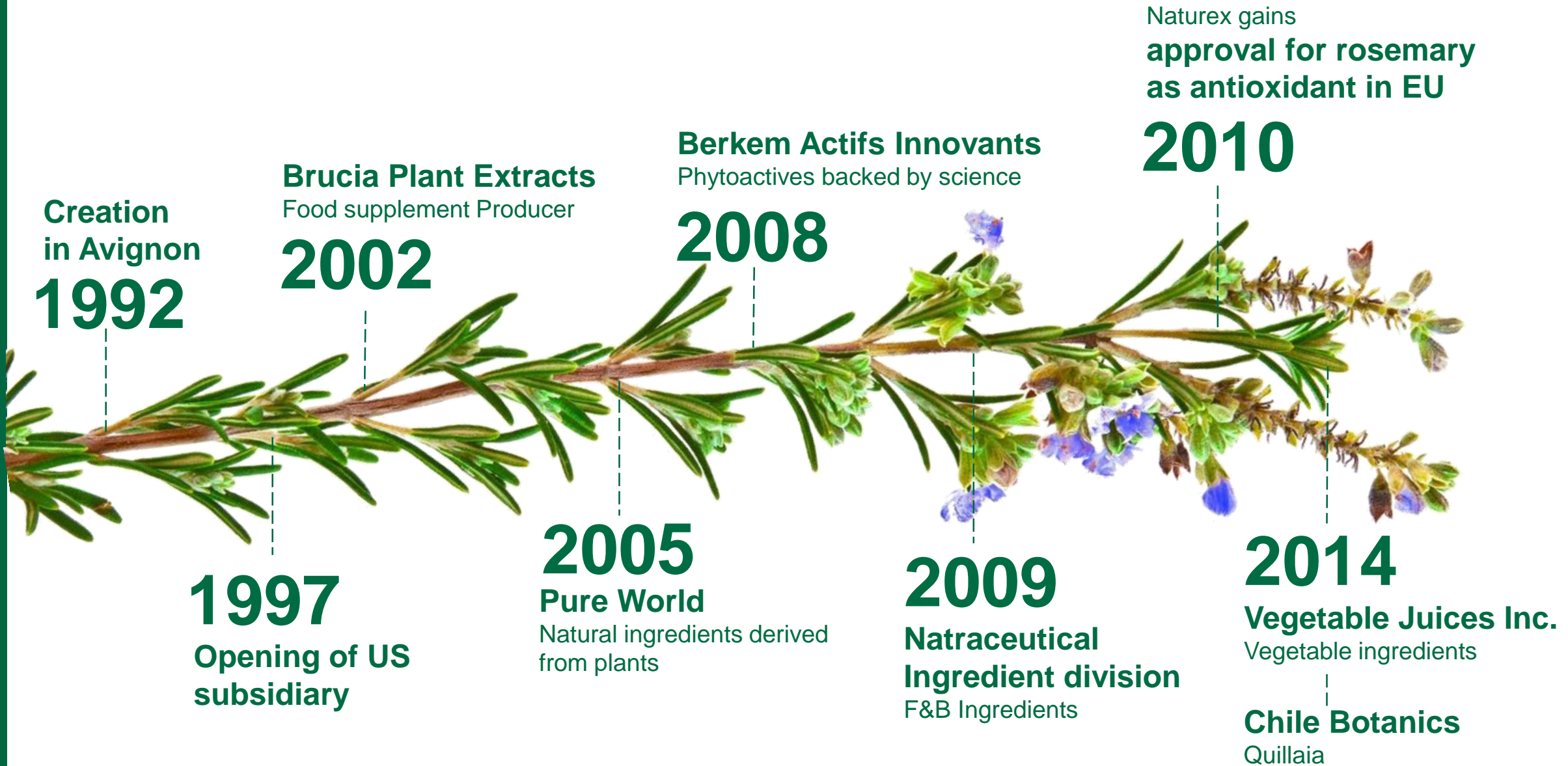
NATUREX 

WHO WE ARE

OVERVIEW
BACKGROUND
EXPERTISE

A GROWING COMPANY

NATUREX 



A LISTED INTERNATIONAL COMPANY

1992 Creation
in Avignon



400m€
in turnover
in 2015



>1,700
Employees
worldwide

OUR LOCATIONS



8
Purchasing
Offices



15
Manufacturing
sites



Global
Sales
network

OUR MARKETS



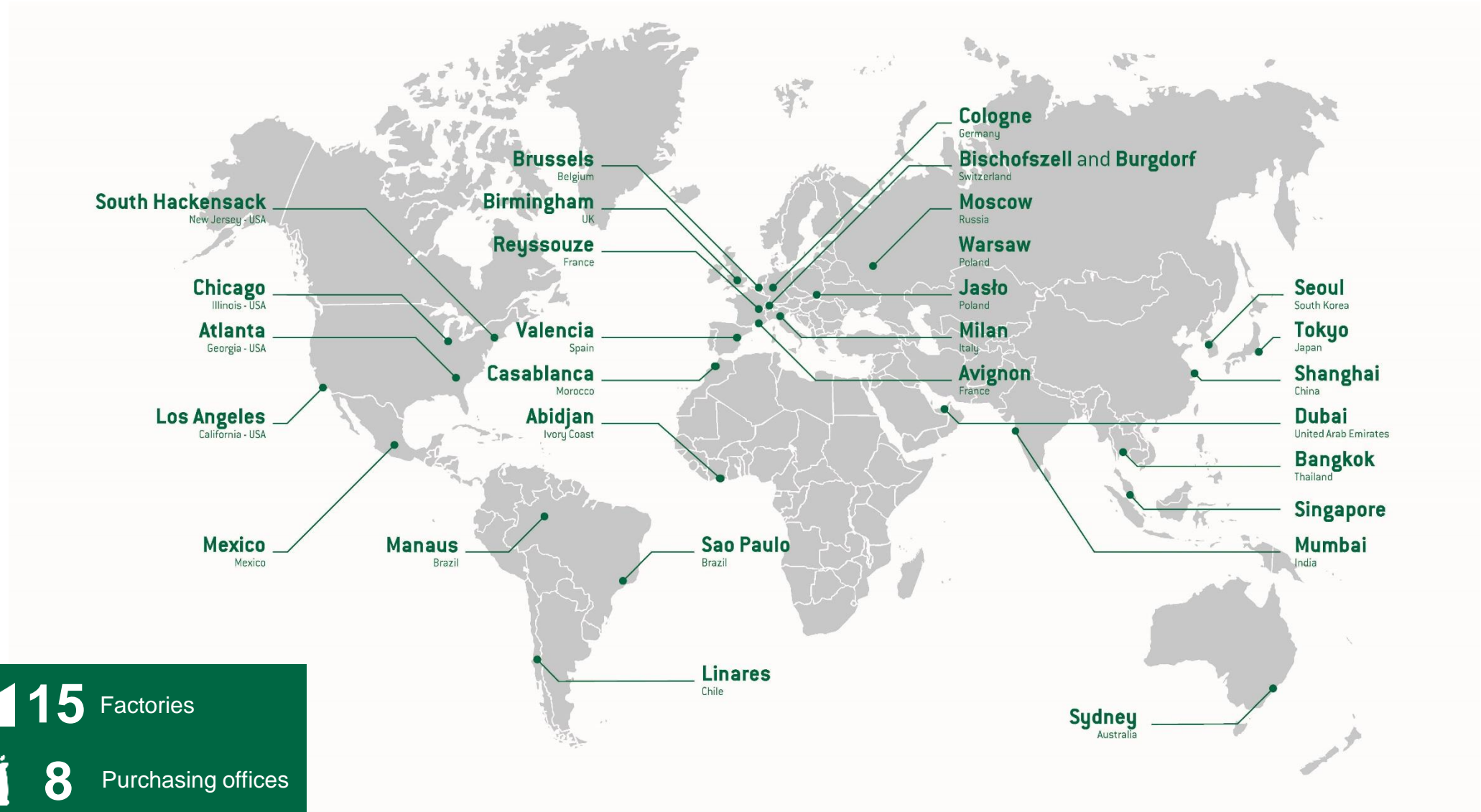
Food & Beverage



Dietary Supplements



Cosmetics



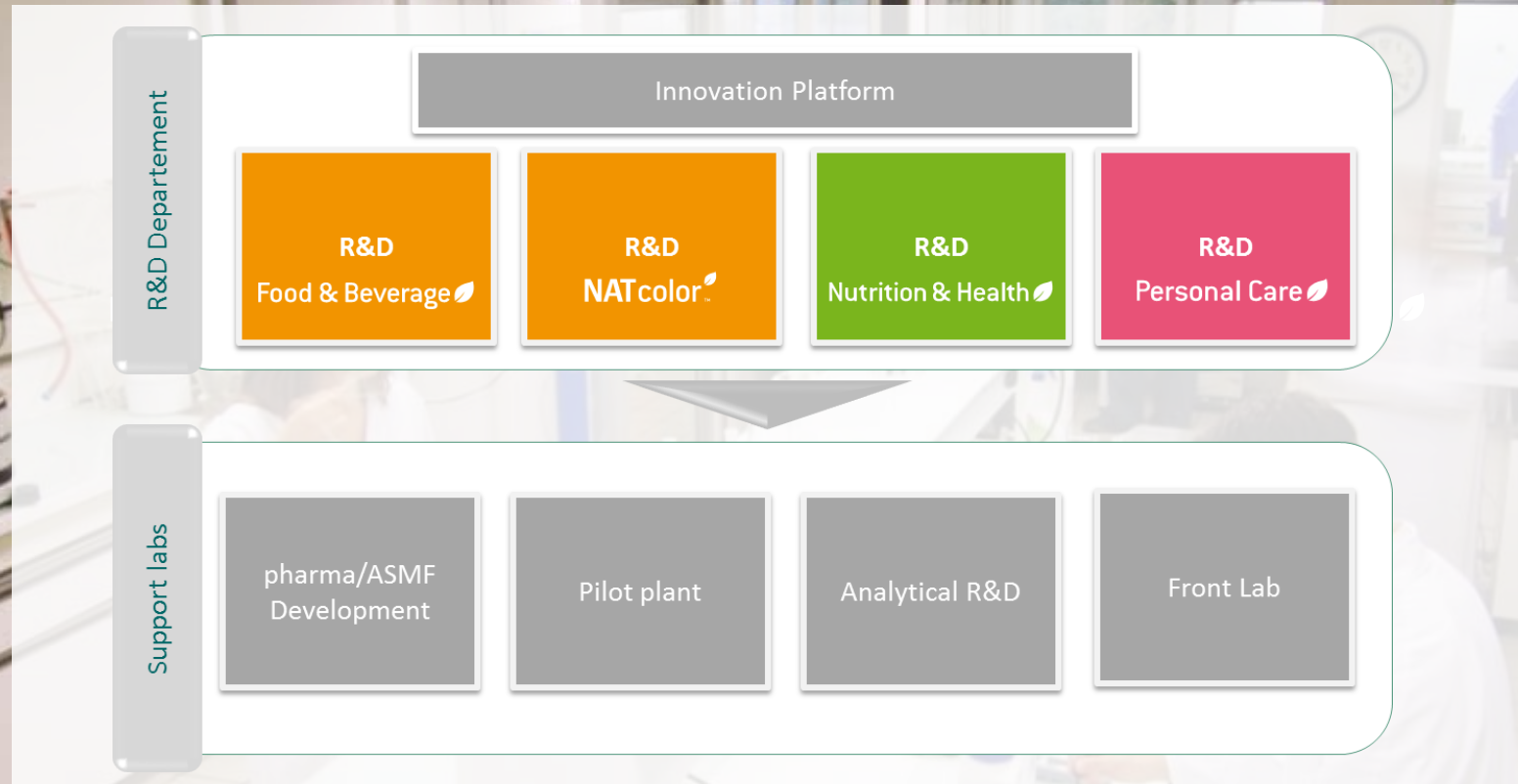
 **15** Factories

 **8** Purchasing offices



WHAT WE DO

Strong R&D, a pillar for innovation



75

17 Ph. D
30 Engineers
28 Technicians

2,000

Developments/
year

5 to 10

Scientific
publications/year

54

Patent families

NATactiv

A broad range of botanicals carefully selected from around the world to meet market demands for traceable, safe and efficacious extracts fully documented with guaranteed lead-time.

NATlife

9 unique patented high-quality natural ingredients whose innovative nature has been scientifically substantiated.

NATpharma

API (CEP) and excipients for pharmaceutical products with full DMF under CTD format .

NATfruits&greens

A range of fruits, vegetables, greens and superfood with preserved taste, color and restituted nutrients content



Gain access to the botanical gardens from around the world

ID & TRACEABILITY

Naturex offer quality phytoactive, safety and traceability to answer consumers demand

NATUREX 





400 HPTLC methods developed, 300 HPLC methods
Collection of authenticated plant specimens

Naturex Integrated platform for identification

Level 1 : Macroscopy/ microscopy and use of validated botanical standard

Level 2 : Phytochemical levels (HPTLC, HPLC, GC, NMR...)

Level 3 : Process knowledge and impact on profile interpretation



HPTLC

HPTLC material available



CAMAG Automatic TLC Sampler 4



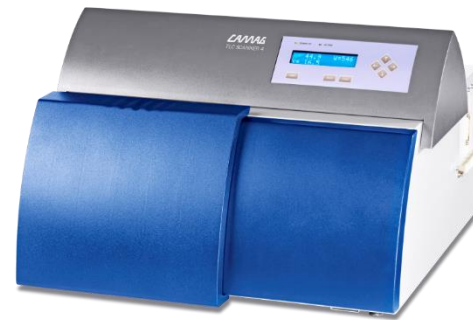
CAMAG Automatic Developing Chamber 2



CAMAG TLC-MS Interface 2



CAMAG TLC Visualizer



CAMAG TLC Scanner 4



Identification Pack

Ref:

Purpose: To provide testing methods used to ensure the proper identification of *Coffea canephora* Pierre ex A. Froehner (syn. *Coffea robusta* L. Linden) Bean (or seed) raw material and extract. Combining and interpreting the multiple testing results and analytical data to guarantee the botanical identification of:

RAW MATERIAL	TEST	METHOD	RESULTS	
	RM1	Macroscopy	Visual	✓ COMPLIANT
	RM2	DNA	External Lab	✓ COMPLIANT
	RM3	HPTLC	QC-SOP-375	✓ COMPLIANT

EXTRACT	TEST	METHOD	RESULTS	
	E1	HPTLC	QC-SOP-375	✓ COMPLIANT
	E2	HPLC	CQ-MO-431	✓ COMPLIANT

Conclusion: Using analytical methods of examination such as Macroscopy, DNA and HPTLC on the raw material along with HPTLC and HPLC on the extract compared with an authenticated reference material ensures the identity of

RM1. Taxonomy / Macroscopic Identification certificate of the raw material: Green coffee bean

Typical batch of raw material used to produce:
Raw Material Name & Ref: Green coffee bean -
Raw Material Batch: A142/003/A15

Bibliographical references: Tropicos.org. Missouri Botanical Garden. 02 May 2016 <<http://www.tropicos.org/Name/27900017>>

Taxonomy:

Class: Equisetopsida
 Subclass: Magnoliidae
 Superorder: Asteranae
 Order: Gentianales
 Family: Rubiaceae
 Genus: Coffea
 Species: *Coffea canephora*

Plant part: Bean (or seed)

Macroscopic characteristics



The drug consists of green beans. The bean is oval, greenish, 10 to 15 mm long and 6 to 8 mm wide. The dorsal side is round; the ventral side is flattened with a longitudinal groove all along. The bean is tough and odourless. The coffee bean comprises a silver skin, an endosperm, and an embryo. Robusta coffee beans have a smaller and less elliptical shape if compared with Arabica beans.

Conclusion: The raw material batch A142/003/A15 has the characteristic macroscopy of Green coffee bean dried raw material.



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EXTRACT	TEST	METHOD	RESULTS	
	E1	HPTLC	QC-SOP-375	✓ COMPLIANT
	E2	HPLC	CQ-MO-431	✓ COMPLIANT

Conclusion: Using analytical methods of examination such as Macroscopy, DNA and HPTLC on the raw material along with HPTLC and HPLC on the extract compared with an authenticated reference material ensures the identity of

RM2. DNA Identification certificate of the raw material: Green coffee bean

Typical batch of raw material used to produce: SVETOL® – GA501071
 Raw Material Name & Ref: Green coffee bean – 1HAA4016
 Raw Material Batch: A142/003/A15



NSE authentication
 2000 Hilltop Drive, Suite C340
 Richmond, CA 94800 USA
 rso@naturextechnologies.com
 (510) 614-3280

CERTIFICATE OF ANALYSIS

SAMPLE INFORMATION
 Customer: Nature SA
 Sample #: 1HAA4016/A142/003/A15
 Description: Green coffee robusta seed
 Species: *Coffea canephora* (syn. *Coffea robusta*)
 Report #: 03-724-1
 Received Date: 04/22/2016
 Report Date: 05/03/2016

TEST INFORMATION (see attached Appendix for more detailed information)
 Method: DNA Species Identification SOP AT-SP-276-7
 Instrument: Ion Torrent Personal Genome Machine Next Generation DNA Sequencer
 Reference: Proprietary HERB™ reference DNA sequence database
 Analysis: FMS, TKH, DLG
 Tests: PP-1

RESULTS
 The sample was analyzed using a universal plant DNA test, which identified it as *Coffea canephora*. No other plant species were identified in the sample.

Fig 1. Species Identification Test Results. The Y-axis is the total number of sequences identified for each species; this value is not representative of weight or volume of the species. "Other" category (if applicable) represents sequences not specifically identified.

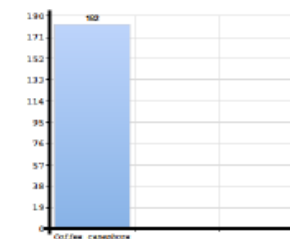


Fig 2. Clean Screen Test Results. The Y-axis is the total number of sequences detected for each species. "LSD" = 30 pg DNA.

No Clean Screen Test was performed.

Verified by:
 Darragh Clancy

The report and its results only related to the specific sample tested and is for exclusive use of the customer named above and shall not be reproduced except in full. All work done in accordance with the Terms and Conditions at www.naturextechnologies.com. © 2016 NSE Authentication. Page 1 of 2

Conclusion: The raw material batch A142/003/A15 is identified by DNA as *Coffea canephora* Pierre ex A. Froehner (syn. *Coffea robusta* L. Linden).



Identification Pack #

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	RM2	DNA	External Lab	✓ COMPLIANT
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EXTRACT	TEST	METHOD	RESULTS	
	E1	HPTLC	QC-SOP-375	✓ COMPLIANT
	E2	HPLC	CQ-MO-431	✓ COMPLIANT

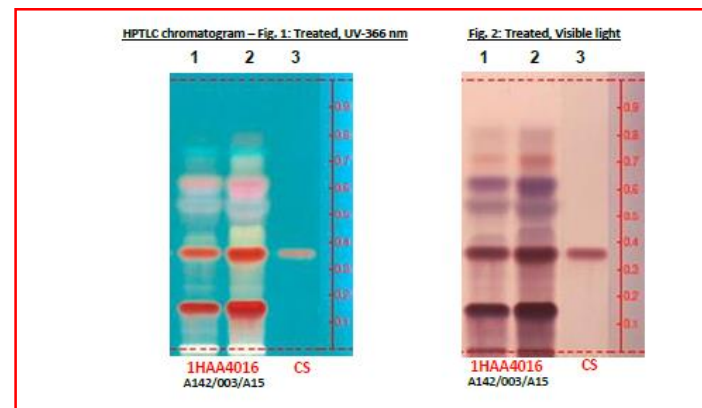
Conclusion: Using analytical methods of examination such as Macroscopy, DNA and HPTLC on the raw material along with HPTLC and HPLC on the extract compared with an authenticated reference material ensures the identity of

RM3. HPTLC Identification certificate of the raw material: Green coffee bean

Typical batch of raw material used to produce:
Raw Material Name & Reference: Green coffee bean
Raw Material Batch: A142/003/A15

Method of analysis: QC-SOP-375

Sample preparation: Extraction of 20 g of the powdered Green coffee bean raw material in 100 ml of tBME and saponification with 50 ml of 10% ethanol KOH. Then purification by liquid/liquid extraction.



Chemical Standard (CS):
 16-O-Methylcafestol*, 80 ng

Lanes 3 (5 µL), 6 (15 µL)

Tested Raw Material Sample:
 Green coffee bean raw material
 Reference 1HAA4016 - Batch A142/003/A15

Lanes 5 (2 µL), 5 (5 µL)

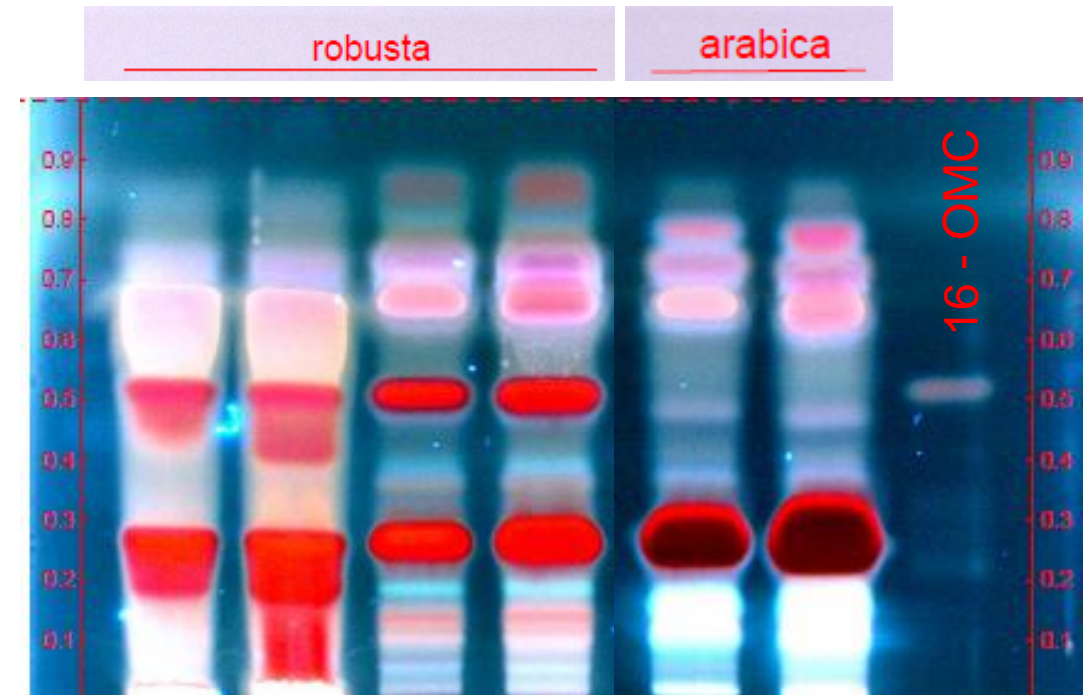
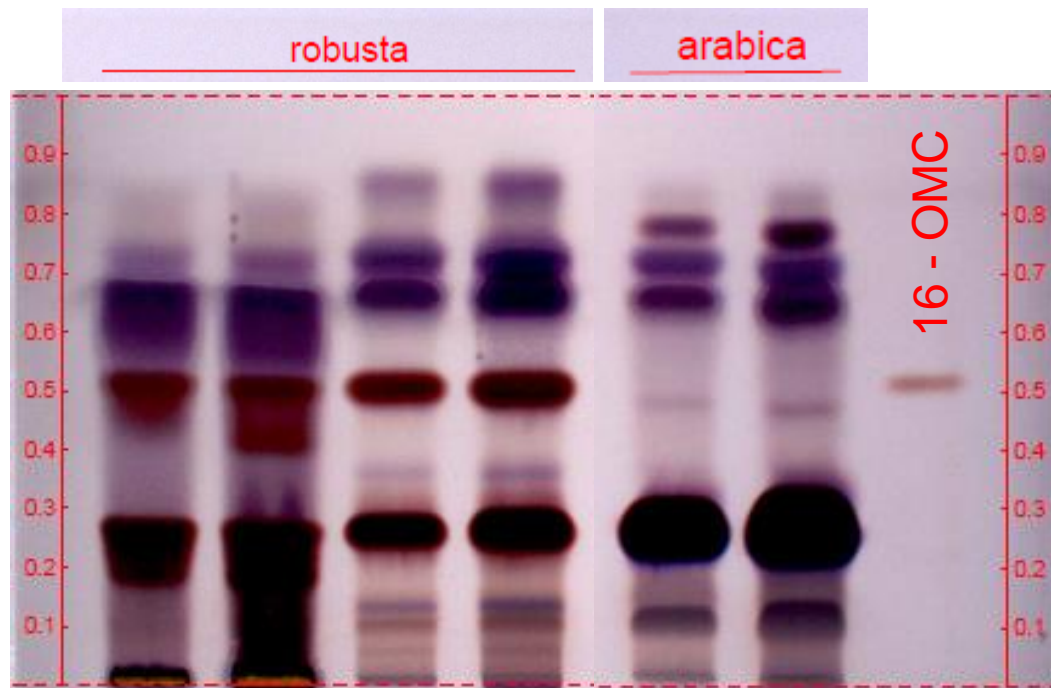
Stationary phase: HPTLC plates silica gel 60 F254. Samples were applied using Camag TLC Sampler 4.
 Mobile phase: tBME / Chloroform 1:1 (v/v).
 Detection: treated with Vanillin / sulfuric acid.

*16-O-Methyl-Cafestol is present in robusta and not in arabica species.

Conclusion: The raw material 1HAA4016 has a typical profile of Green robusta coffee bean (16-O-Methyl-Cafestol is present in robusta and not in arabica species)

HPTLC method

Distinction arabica – robusta



Sample preparation : extraction in tBME, saponification and liquid-liquid separation then dilution in 100 % MeOH

Mobile phase: tBME / chloroform (1:1) (v:v)

Detection : Vanillin

Methanolic / Ethanolic extract only



Identification Pack

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	RM3	HPTLC	QC-SOP-375	✓ COMPLIANT

EXTRACT	TEST	METHOD	RESULTS	
	E1	HPTLC	QC-SOP-375	✓ COMPLIANT
	E2	HPLC	CQ-MO-431	✓ COMPLIANT

Conclusion: Using analytical methods of examination such as Macroscopy, DNA and HPTLC on the raw material along with HPTLC and HPLC on the extract compared with an authenticated reference material ensures the identity of

E1. HPTLC Identification certificate of the botanical extract

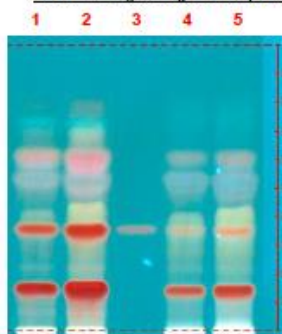
Botanical extract name & reference:

Botanical extract batch: A174/019/A1

Method of analysis: QC-SOP-375

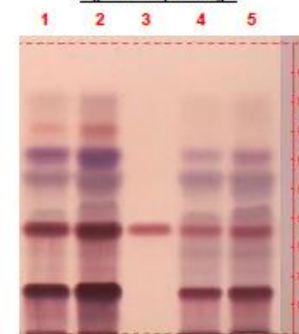
Sample preparation: Extraction of 10 or 20 g of the powdered Green coffee bean in 100 ml of tBME and saponification with 50 ml of 10% ethanol KOH. Then purification by liquid/liquid extraction.

HPTLC chromatogram – Fig. 1: Treated, UV-366 nm



BRM CS
A142/003/A15 A174/019/A15

Fig. 2: Treated, Visible light



BRM CS
A142/003/A15 A174/019/A15

Botanical Reference Material (BRM):

Green coffee bean, *Coffea canephora* Pierre ex A. Froehner (syn. *Coffea robusta* L. Linden)
 Authenticated Reference: 1HAA4016 - Batch A142/003/A15

Chemical Standard (CS):

16-O-Methylcafestol*, 80 ng

Tested Botanical Extract Sample:

Lanes 1 (2 µl), 2 (3 µl)

Lane 3 (10 µl)

Lanes 4 (3 µl), 5 (10 µl)

Stationary phase: HPTLC plates silica gel 60 F254. Samples were applied using Camag TLC Sampler 4.

Mobile phase: tBME / Chloroform 1:1 (v/v).

Detection: treated with Vanillin / Sulfuric acid.

*16-O-Methyl-Cafestol is present in robusta and not in arabica species.

Conclusion: The HPTLC profile of A174/019/A15 is consistent with the chromatographic profile of an extract derived from Green robusta coffee bean.



Identification Pack

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	RM2	DNA	External Lab	✓ COMPLIANT
	RM3	HPTLC	QC-SOP-375	✓ COMPLIANT

EXTRACT	TEST	METHOD	RESULTS	
	E1	HPTLC	QC-SOP-375	✓ COMPLIANT
	E2	HPLC	CQ-MO-431	✓ COMPLIANT

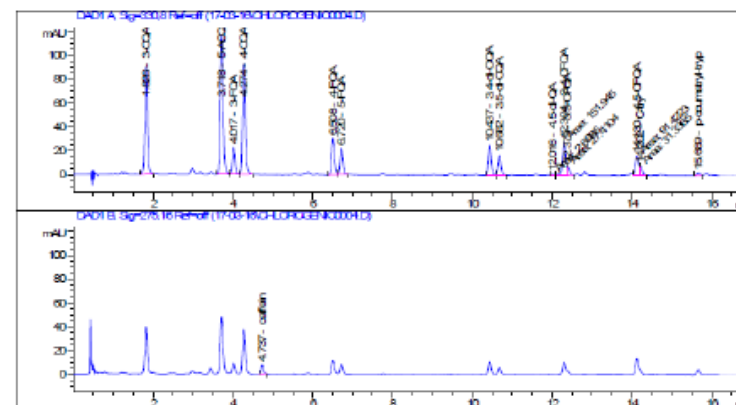
Conclusion: Using analytical methods of examination such as Macroscopy, DNA and HPTLC on the raw material along with HPTLC and HPLC on the extract compared with an authenticated reference material ensures the identity of

E2. HPLC Identification certificate of the botanical extract:

Botanical extract name & reference:
Botanical extract batch: A174/019/A15

Method Analysis: CQ-MO-431

Profile of chlorogenic acids in Green coffee bean Extract



Conclusion: The HPLC profile of A174/019/A15 is consistent with the chromatographic profile of an extract derived from Green coffee bean.

Piper methysticum G. Forst. : Kava kava roots



Origin : Pacific islands

Traditional beverage : cold water extraction of underground organs

Properties : relaxing / anxiolytic

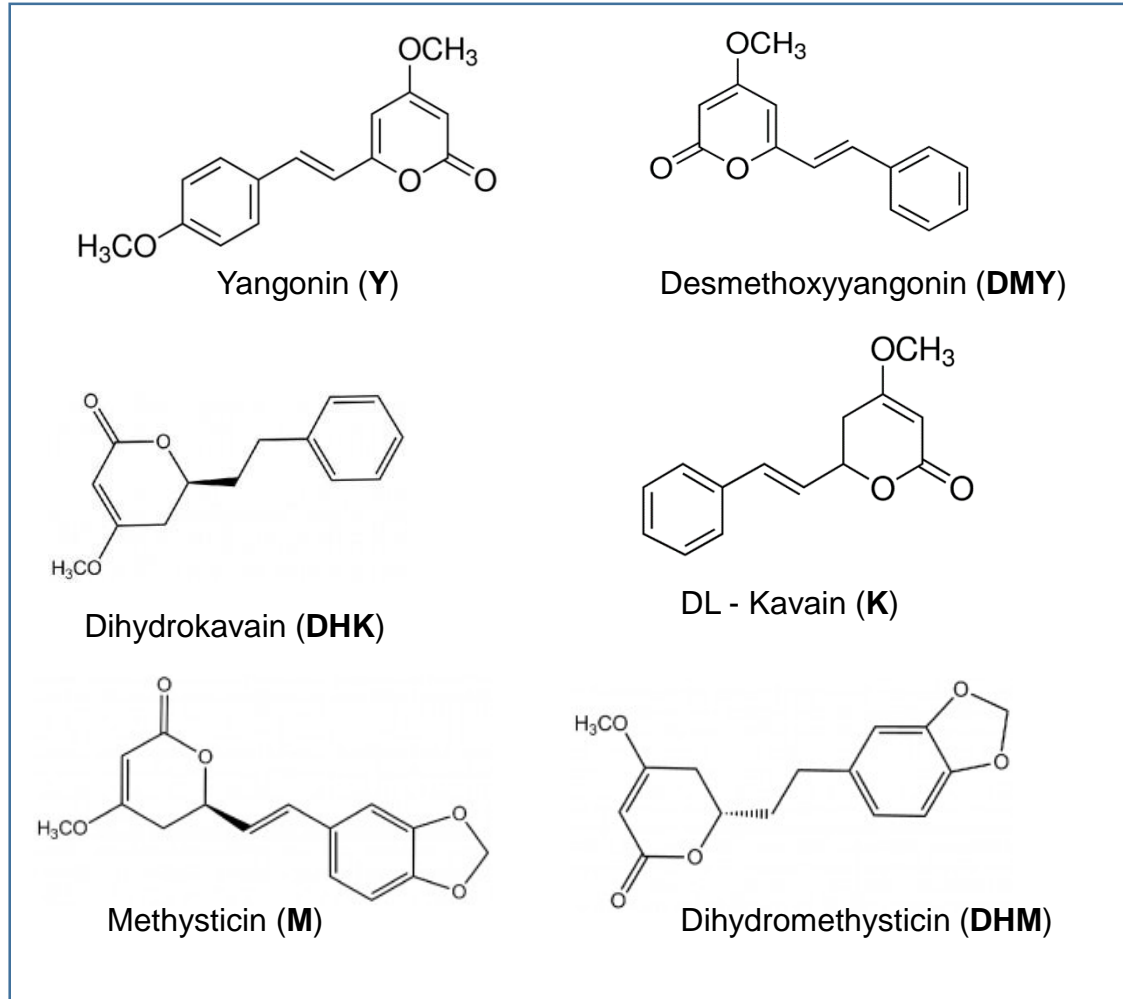
Actives: Kavalactones

Banned from Europe

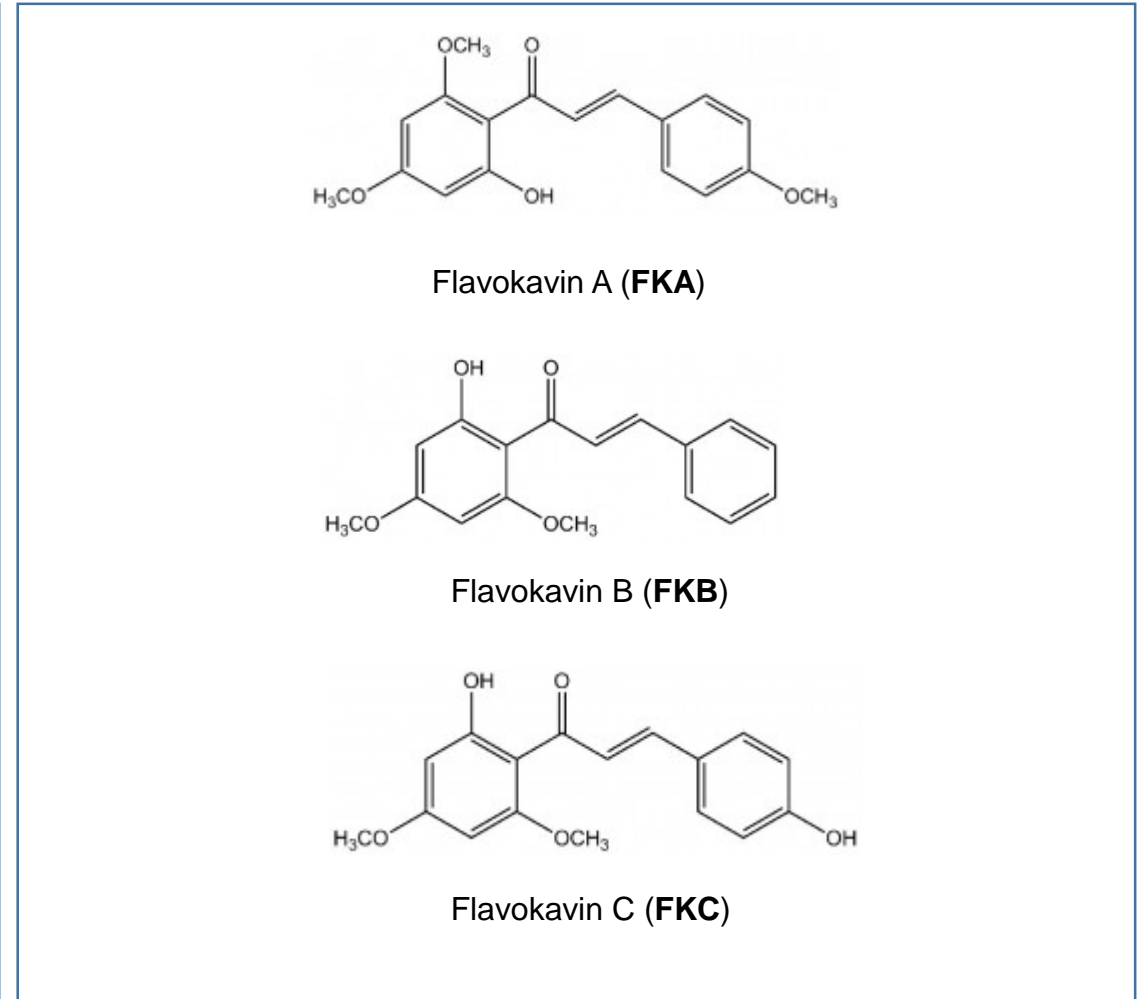
Suspected of liver toxicity → (Flavokavin B ?)

Kava act – Dec 2002 : regulation of the most suitable cultivars : 28 noble cultivars accepted

Piper methysticum G. Forst. : Kava kava roots

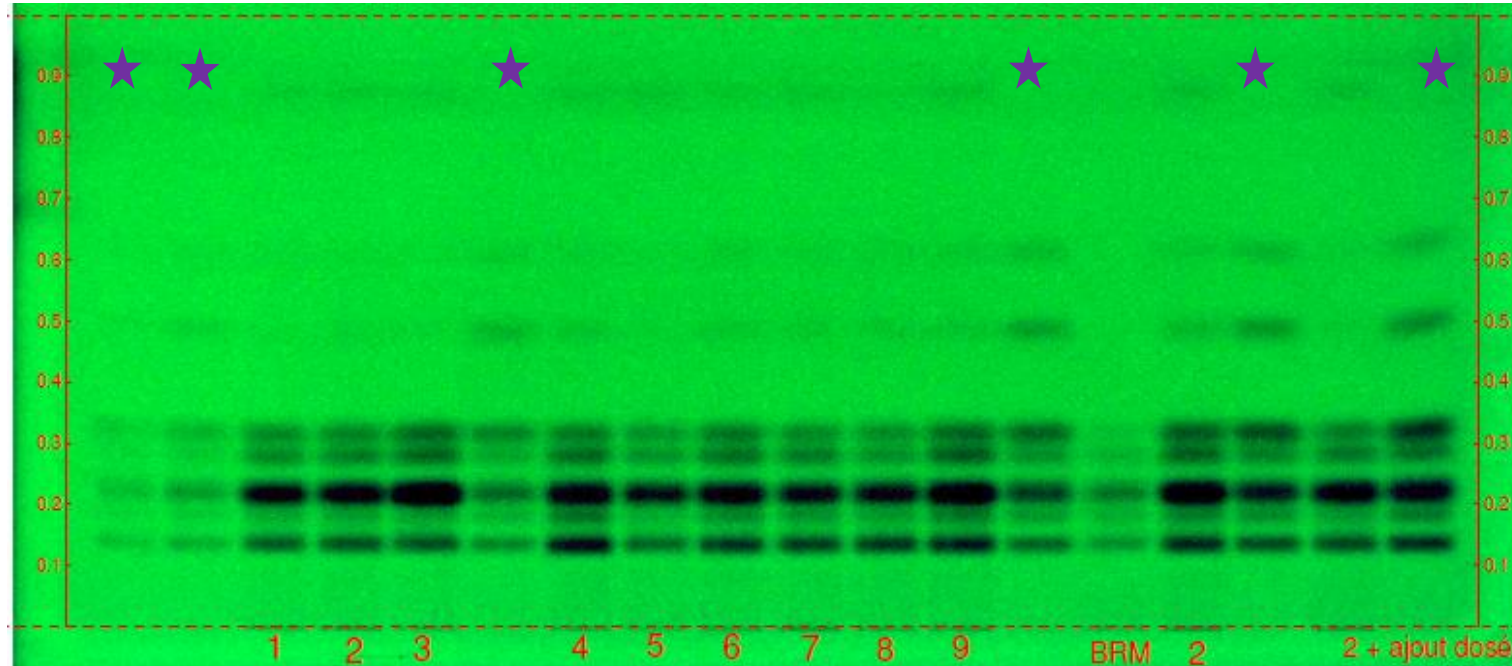


Kavalactones (KL)



Flavokavins (FK)

Piper methysticum : Kava kava roots



★ Standard range

Sample preparation : grounded, dried 24h @ 65 °C, then extract in acetone

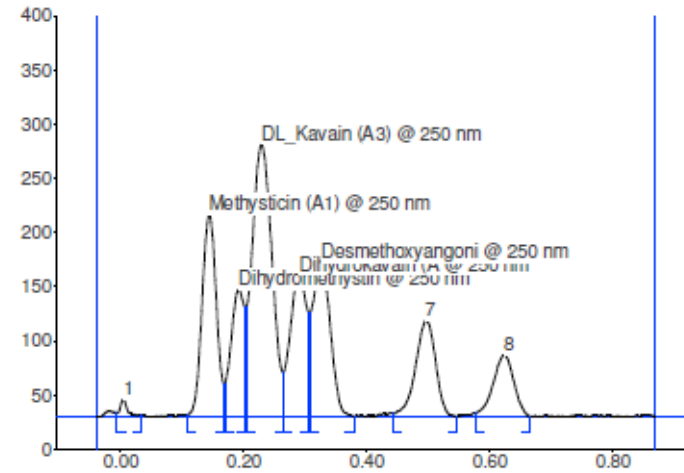
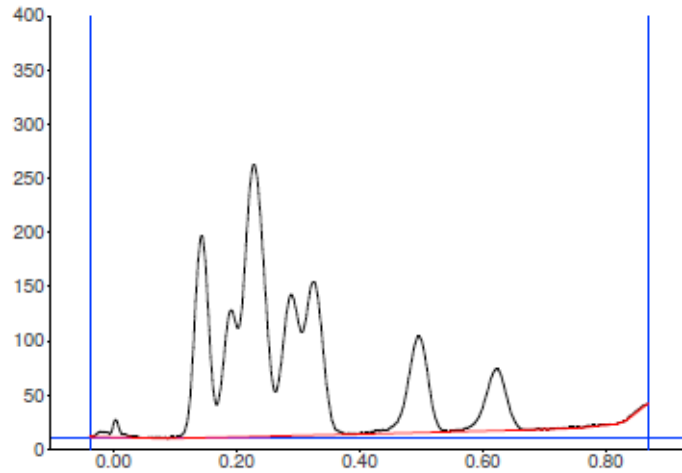
Mobile phase : Hexane: dioxane (8:2) (v/v)

Derivatizer : none

Piper methysticum : Kava kava roots

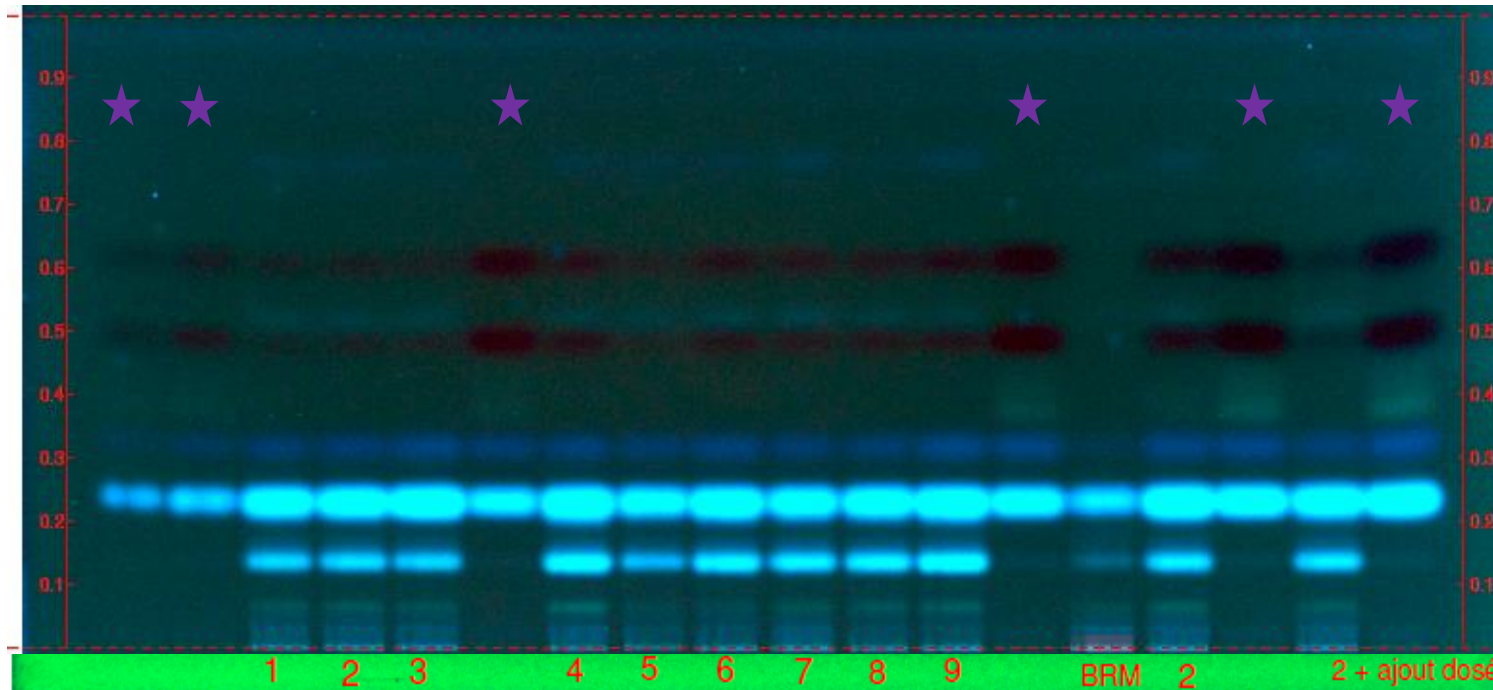
Dosage @ 254 nm

Track 6, ID: Standard3



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	3.5	0.00	16.3	1.64	0.03	1.2	176.8	0.71	unknown *
2	0.11	1.6	0.14	186.4	18.84	0.17	30.2	3906.9	15.60	Methysticin (A1)
3	0.17	30.5	0.19	117.3	11.85	0.20	100.6	2321.0	9.27	Dihydromethystin
4	0.20	101.2	0.23	251.3	25.40	0.26	40.9	7384.7	29.49	DL_Kavain (A3)
5	0.26	41.3	0.29	130.3	13.17	0.31	95.3	3226.6	12.89	Dihydrokavain (A)
6	0.31	96.0	0.33	141.7	14.32	0.38	0.7	3670.2	14.66	Desmethoxyangoni
7	0.44	2.8	0.50	89.2	9.02	0.55	0.3	2614.2	10.44	unknown *
8	0.58	3.6	0.62	57.0	5.76	0.67	0.5	1739.5	6.95	unknown *

Piper methysticum : Kava kava roots



Sample preparation : grounded, dried 24h @ 65 °C, then extract in acetone

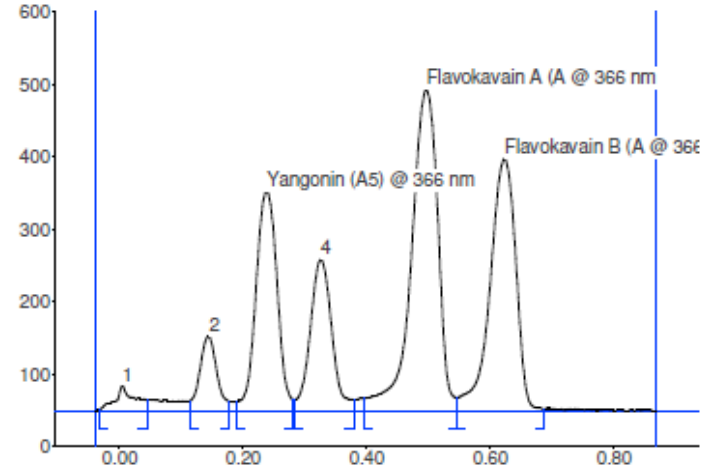
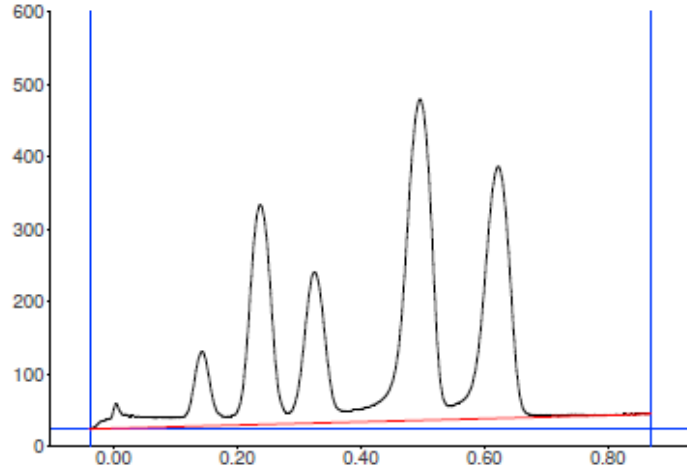
Mobile phase : Hexane: dioxane (8:2) (v/v)

Derivatizer : none

Piper methysticum : Kava kava roots

Dosage @ 366 nm

Track 6, ID: Standard3

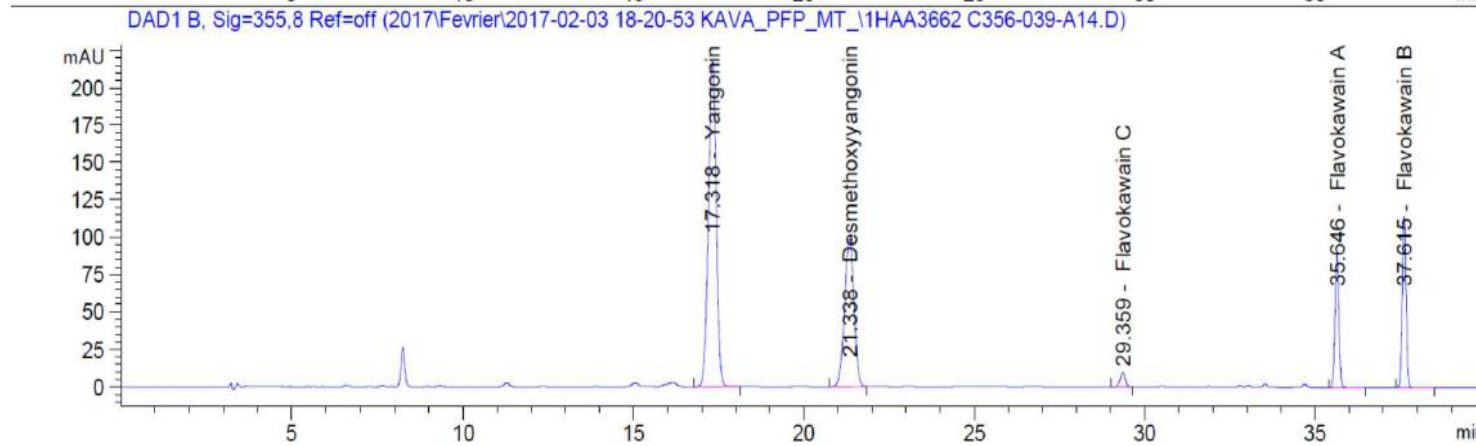
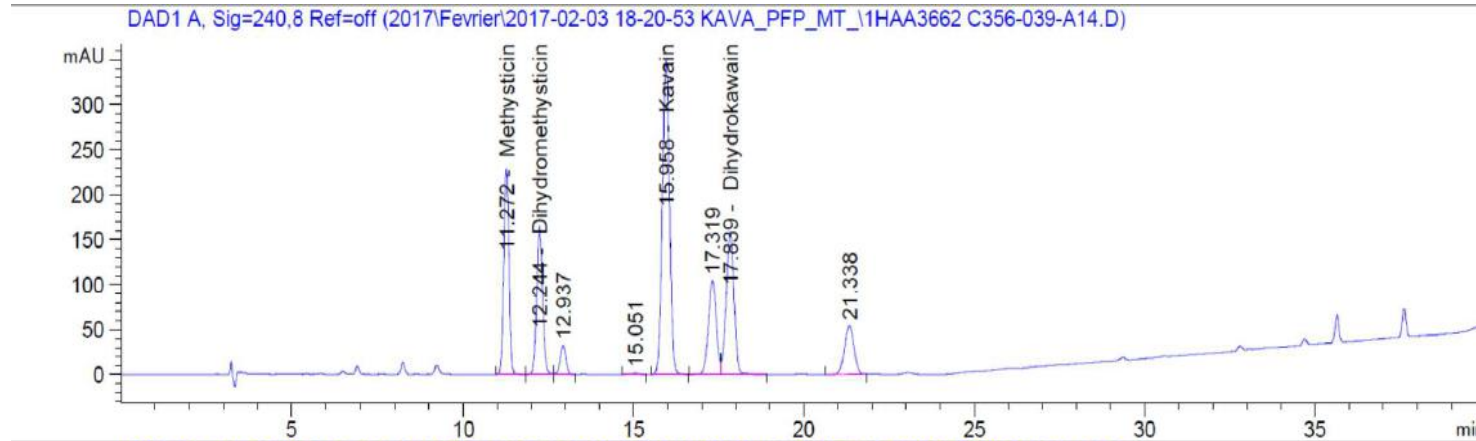


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	1.8	0.00	34.2	2.38	0.04	14.6	969.4	1.96	unknown *
2	0.11	13.6	0.14	103.0	7.16	0.18	12.2	2553.1	5.15	unknown *
3	0.19	12.9	0.24	303.1	21.06	0.28	15.6	9315.8	18.81	Yangonin (A5)
4	0.28	15.6	0.33	208.3	14.47	0.38	15.2	6553.8	13.23	unknown *
5	0.40	17.0	0.50	443.0	30.78	0.55	18.2	17121.1	34.57	Flavokavain A (A
6	0.55	18.3	0.62	347.6	24.15	0.69	2.1	13014.8	26.28	Flavokavain B (A

+ Flavokavain C

Piper methysticum : Kava kava roots

Cross results with HPLC-DAD



Piper methysticum : Kava kava roots

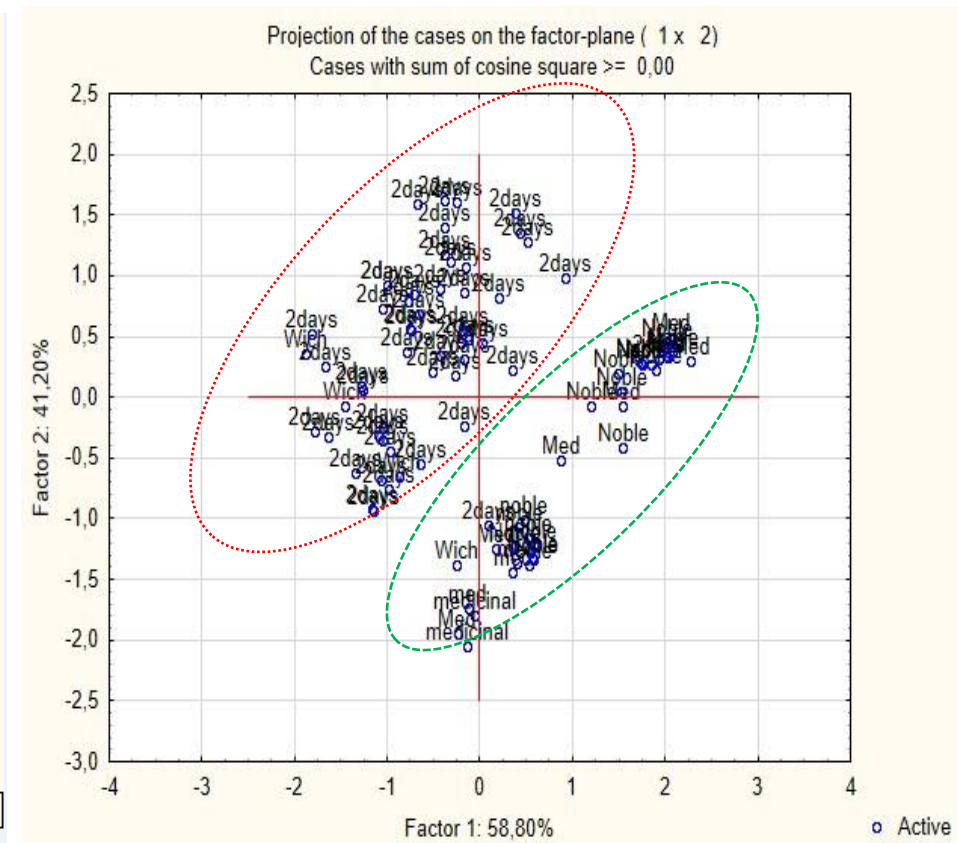
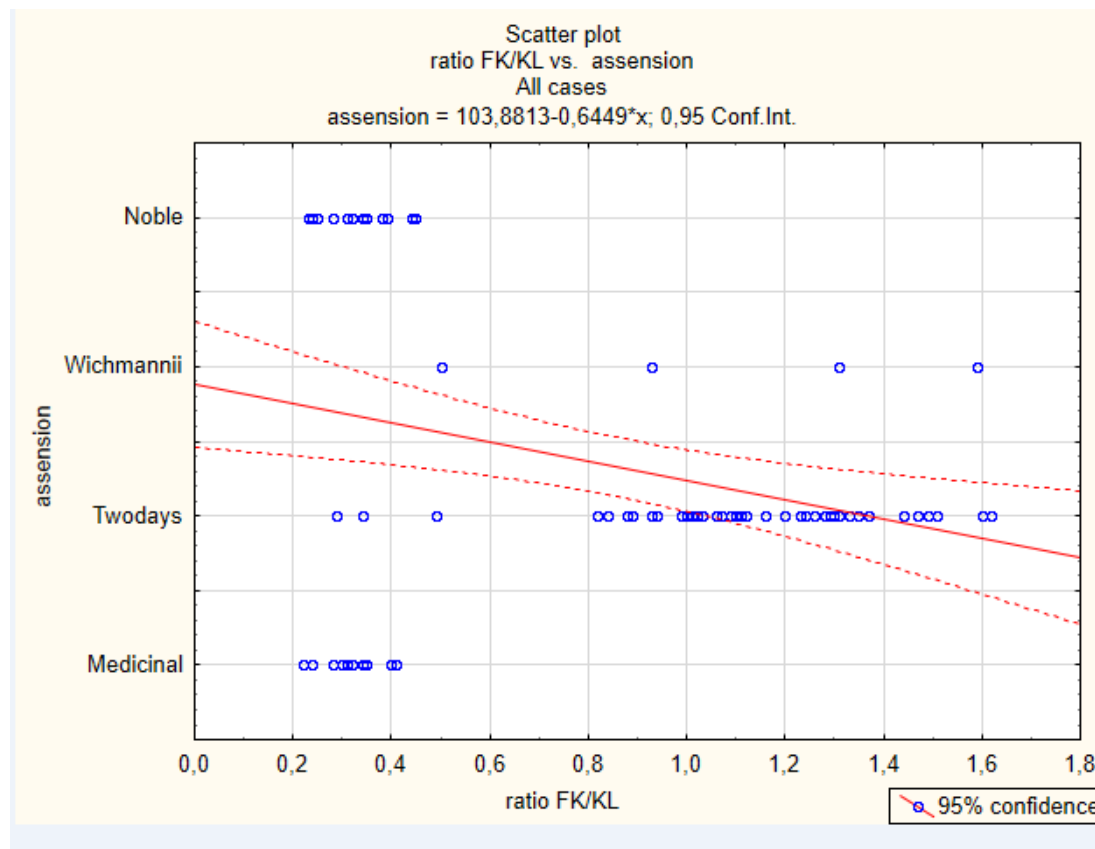
Cross results with HPLC-DAD



RM	Lebot et. al (2014)								Results (HPLC/HPTLC)								Probable Cultivar according to Lebot et al. criteria
	Noble				Two days				FKA / (Y+DMY)		FKB / (Y+DMY)		FKC/(Y+DMY)		FK/KL		
	FKA / (Y+DMY)	FKB/ (Y+DMY)	FKC/ (Y+DMY)	FK/KL	FKA / (Y+DMY)	FKB/ (Y+DMY)	FKC/ (Y+DMY)	FK/KL	FKA / (Y+DMY)	FKB / (Y+DMY)	FKC/(Y+DMY)	FKC/(Y+DMY)	FKC/(Y+DMY)	FKC/(Y+DMY)	FK/KL	FK/KL	
1									0,08	0,07	0,12	0,13	0,01	0,04	0,21	0,24	Noble
2									0,09	0,07	0,13	0,13	0,01	0,05	0,24	0,25	Noble
3									0,06	0,05	0,08	0,08	0,01	0,03	0,15	0,16	Noble
4	0,10 (+/- 0,04)	0,09 (+/- 0,04)	0,10 (+/- 0,04)	0,29 (+/- 0,10)	0,39 (+/- 0,10)	0,39 (+/- 0,12)	0,24 (+/- 0,07)	1,02 (+/- 0,28)	0,15	0,11	0,15	0,15	0,02	0,06	0,21	0,31	Undefined (maybe Noble, Not two days)
5									0,08	0,06	0,11	0,12	0,01	0,05	0,21	0,23	Noble
6									0,14	0,10	0,20	0,19	0,02	0,05	0,36	0,35	Undefined (maybe Noble, Not two days)
8									0,17	0,14	0,19	0,21	0,02	0,07	0,38	0,41	Undefined (maybe Noble, Not two days)
9									0,13	0,11	0,20	0,21	0,02	0,06	0,35	0,38	Undefined (maybe Noble, Not two days)

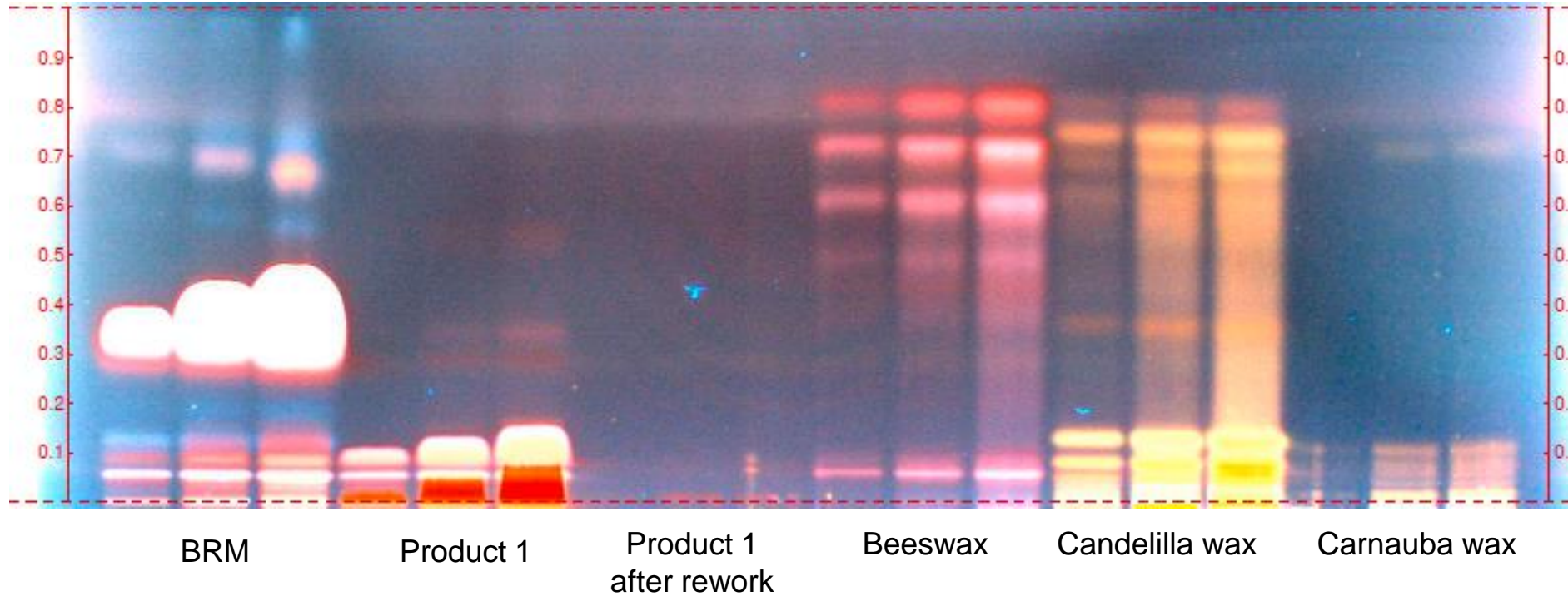
Piper methysticum : Kava kava roots

Statistical analysis



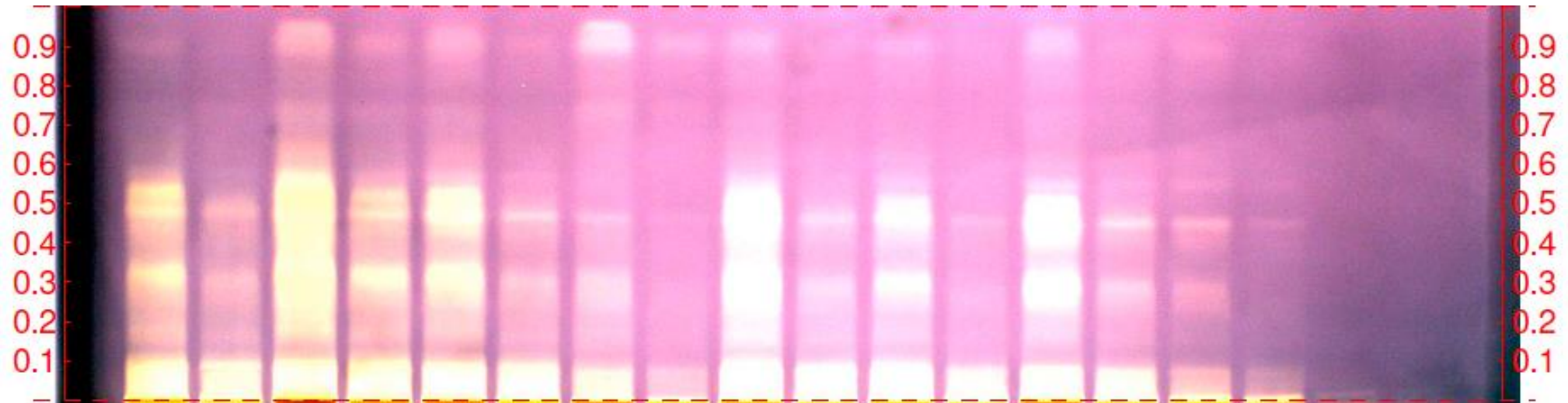
Investigation during product development

Research of insoluble



Investigation during product development

Activity testing



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