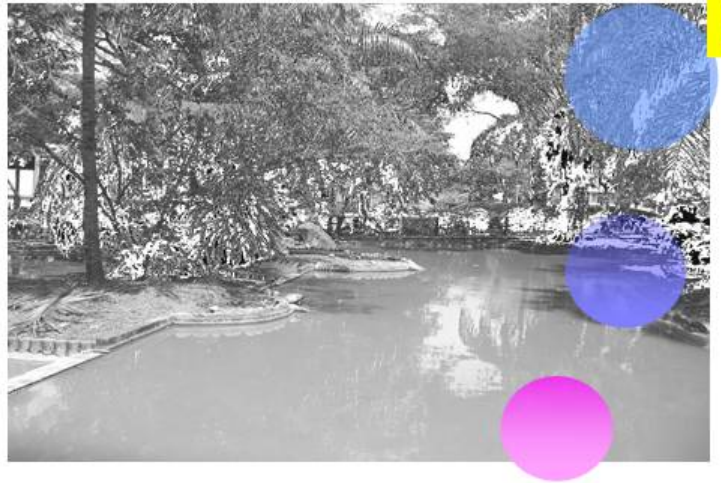
The Many Roles of *Separation Science* in Developing Countries: the Colombian Case

Prof. Dr. *Elena E. Stashenko*

Industrial University of Santander
Bucaramanga, Colombia, S.A.



Industrial University of Santander
Bucaramanga, Colombia, South America

South America

Area: **2.070.408 km²**

Brazil (1), Argentina (2), Perú (3), Colombia (4)

Population: **ca. 41.000.000**

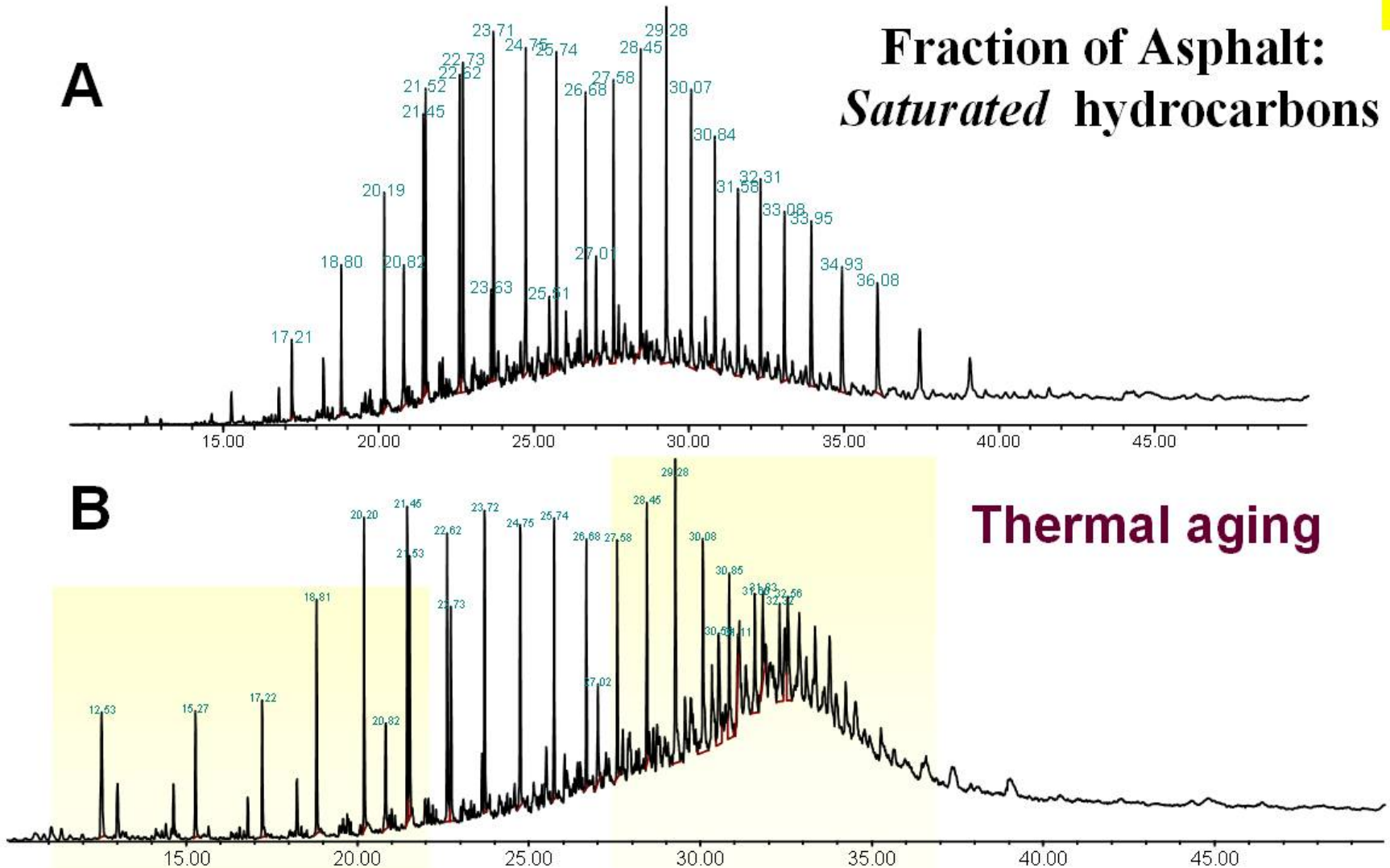
Capital: **Bogotá**

- Minerals
- Gold
- Silver
- Platinum
- Nickel
- Emeralds

"El Dorado"

- **Petroleum**
- **Natural gas**

Fraction of Asphalt: *Saturated* hydrocarbons



Comparison of asphalt saturated hydrocarbon fractions before (**A**) and after (**B**) thermal aging. GC-FID, DB-1 (30 m)

A

Fraction of Asphalt
***Saturated* hydrocarbons**

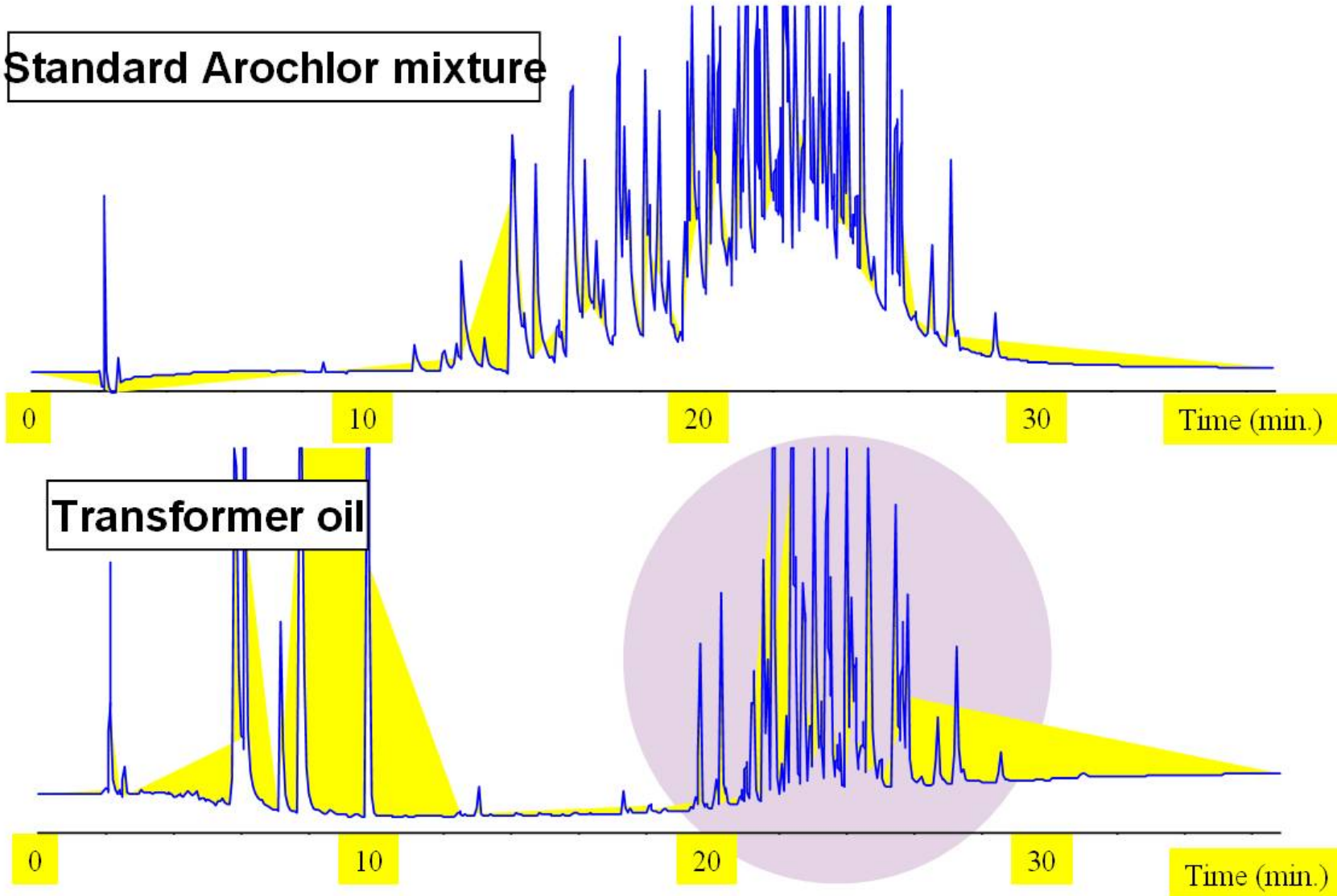
B

Aging under pressure

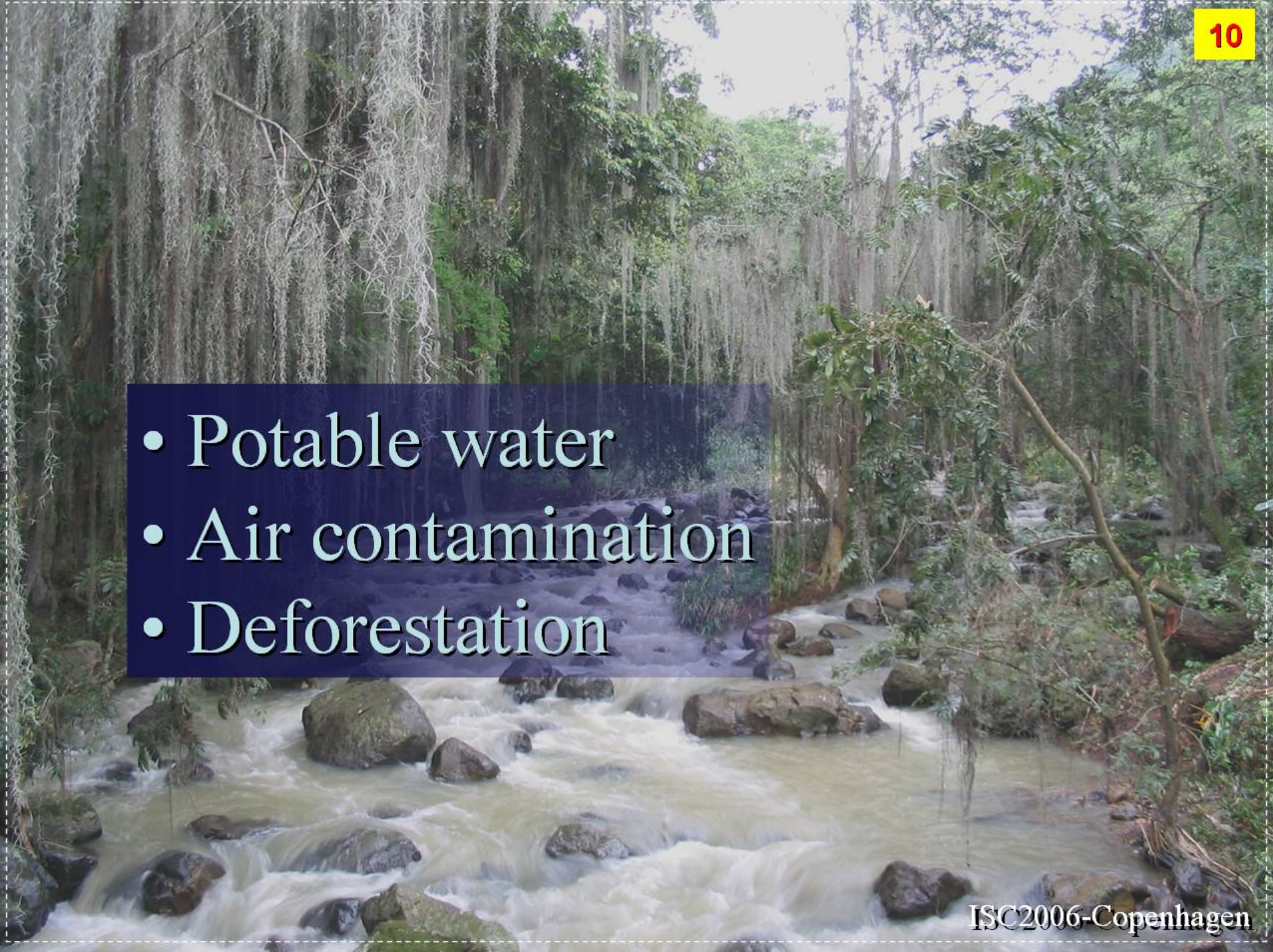
Comparison of asphalt saturated fractions before (A) and after (B) aging under applied pressure. **GC-FID. DB-1 (30 m)**

• Environment

- Pesticides
- Glyphosate
- PCBs
- Hydrocarbons
- Hg



GC-ECD. Column HP-5 (30 m x 0.25 mm x 0.25 µm).



- Potable water
- Air contamination
- Deforestation

• Biodiversity

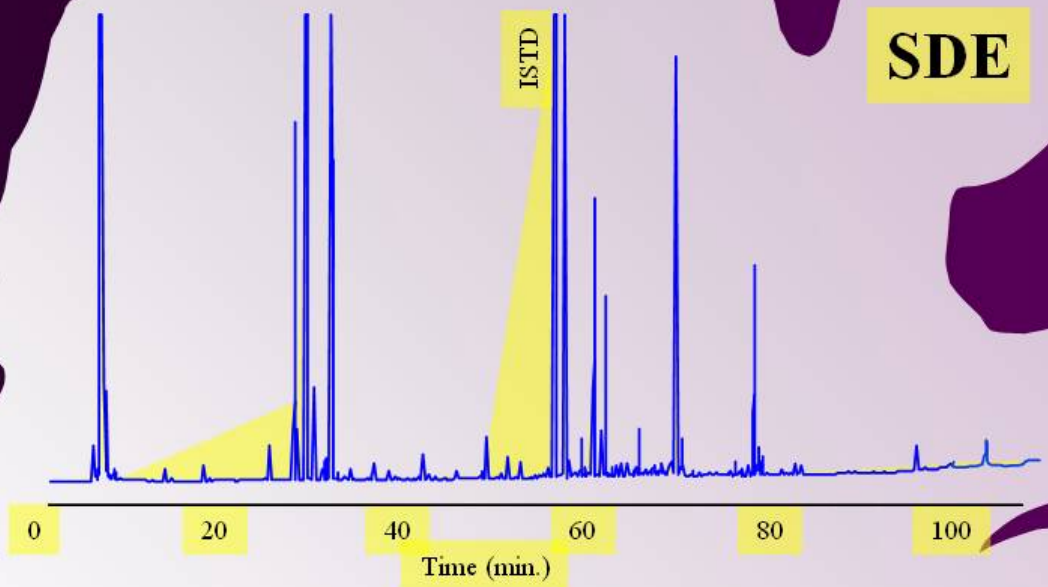
Colombia:

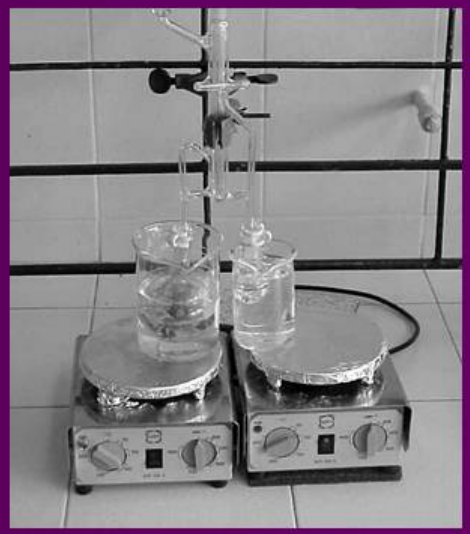
- 10% of total vegetal species on 0.7% of Earth's surface
- Second world place in biodiversity (after Brazil)

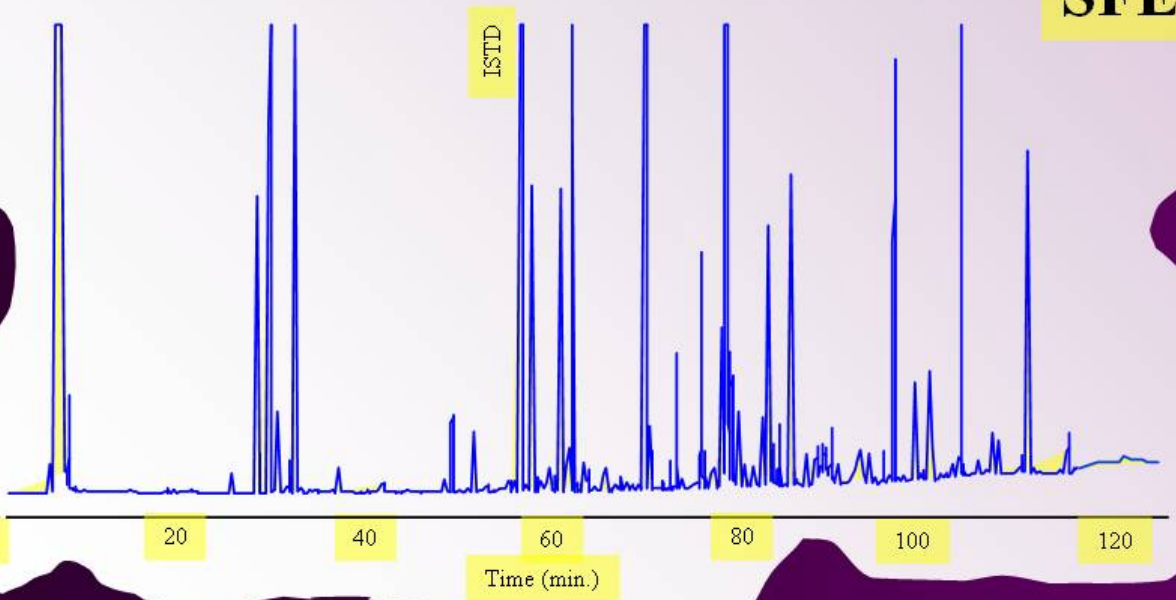
Spilanthes americana (Mutis) Hieron (Fam. *Compositae*), commonly called in Colombia "*guaca*", "*guasca*" or "*quemadora*", is native to South America, and is used widely in Colombian cuisine.

Extracts from *S. americana* have been shown to possess antiseptic and analgesic effects, and insecticidal activity against adult mosquitoes, several leaf-eating insects and houseflies. The plant produces a slight burning, paralytic effect on the tongue.

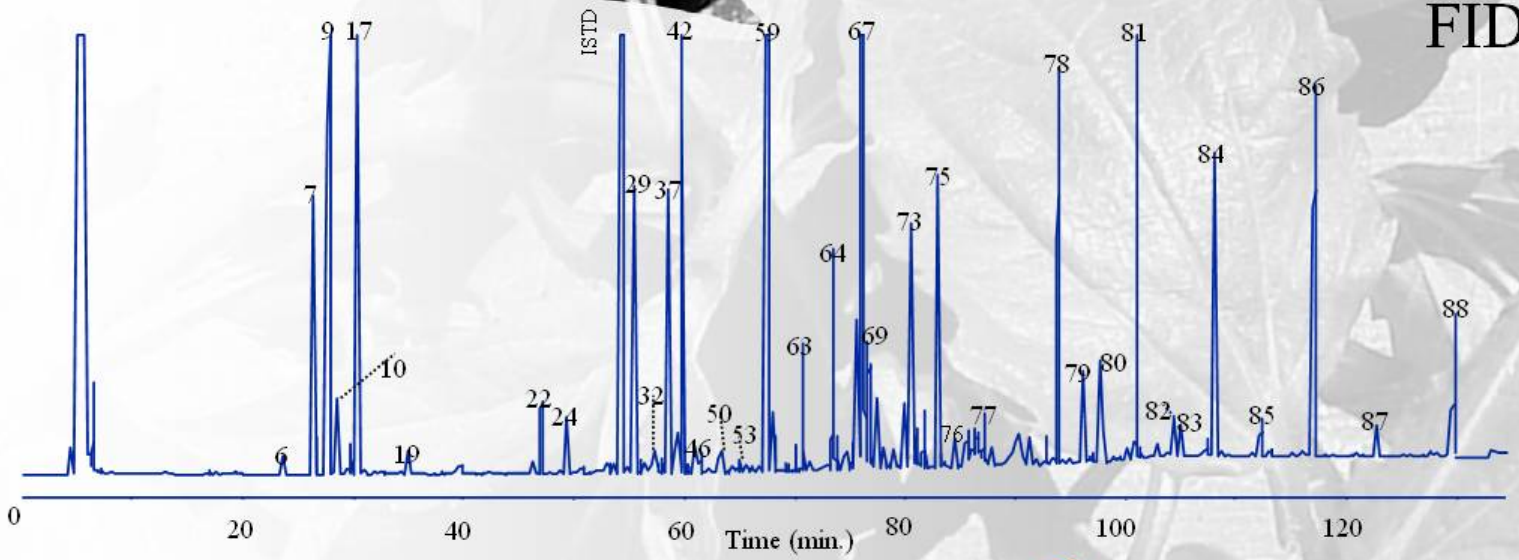
The extracts from *S. americana* flowers also have found application in the treatment of *Herpes simplex* and *Herpes zoster* (dangerous virus infection).

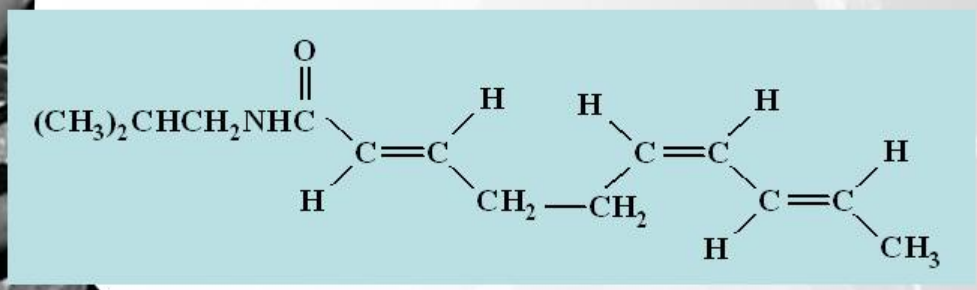

SDE
ISTD
0
20
40
60
80
100
Time (min.)

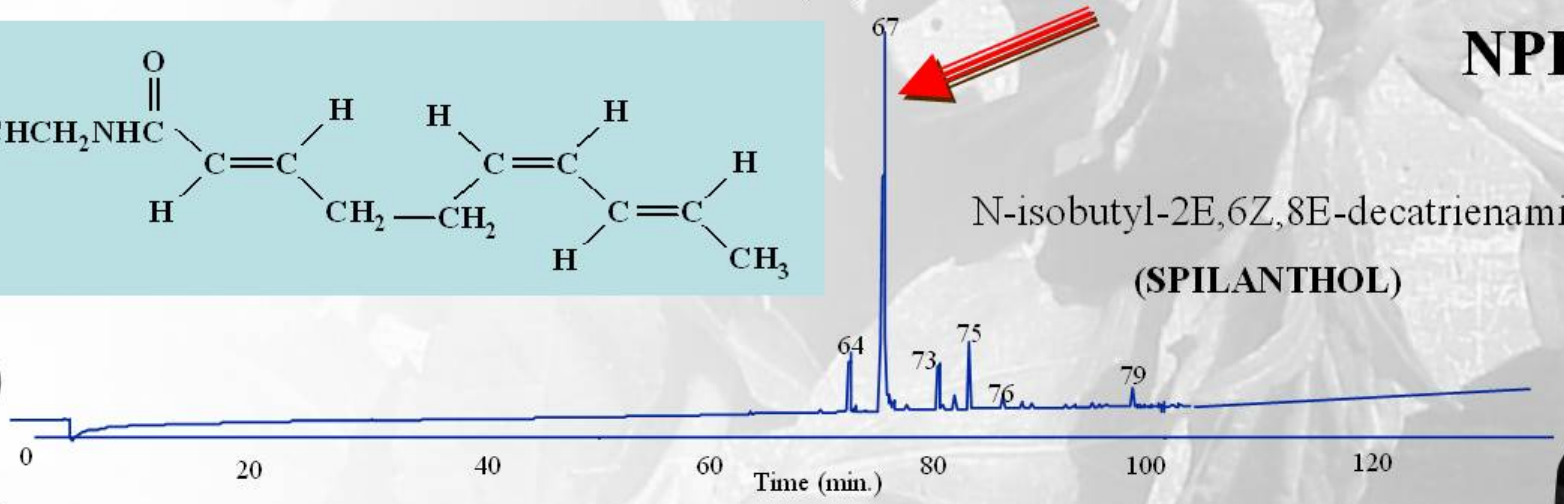



SFE
ISTD
0
20
40
60
80
100
120
Time (min.)

High Pressure
Soxhlet Extractor
J&B Scientific







N-isobutyl-2E,6Z,8E-decatrienamide

(SPILANTHOL)

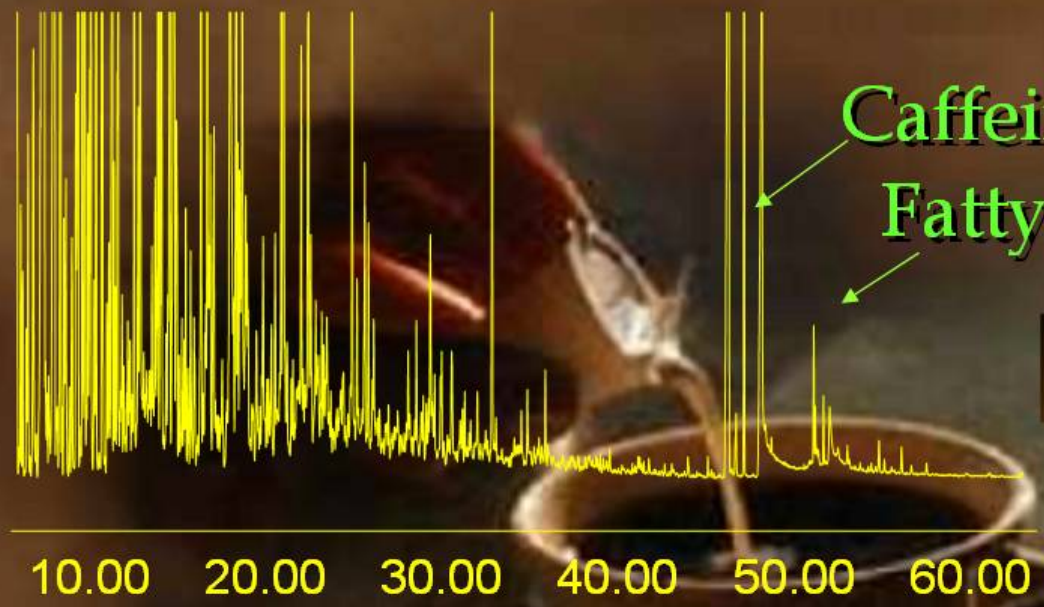
E. E. Stashenko *et al.*, *J. Chromatogr. A*, 1996, 752, 223.

Although Colombia is extremely rich in aromatic plants, which we have been studying intensively, paradoxically, in the country, there is no established industry for essential oil production…

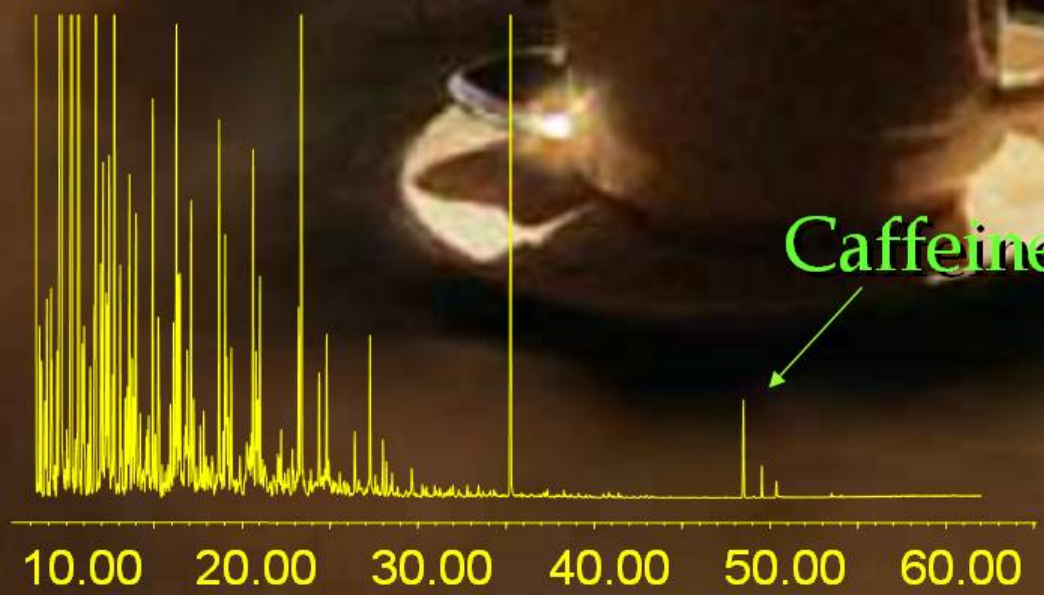
Essential oil composition = *f* (*x*)

Elena E. Stashenko *et al.*:
J. Chromatogr. A, **2004**, 1025, 105-113.
J. Chromatogr. A, **2004**, 1025, 99-103.
J. Flav. Fragr, **2002**, 17, 380.
J. Biochem. Biophys. Methods, **2000**, 43, 379.
J. Chromatogr. A, **1996**, 752, 223.
J. High Resol. Chromatogr., **1996**, 19 (6), 353.
J. Microcolumn Sep., **1995**, 7(2), 117.
J. High Resol. Chromatogr., **1995**, 18(2), 101.
J. High Resol. Chromatogr., **1994**, 17(9), 643.
J. High Resol. Chromatogr., **1993**, 16(7), 441.

- Coffee
- Cocoa
- Banana
- Fruits
- Cereals
- Palm oil
- Latex
- Tobacco
- Sugar cane

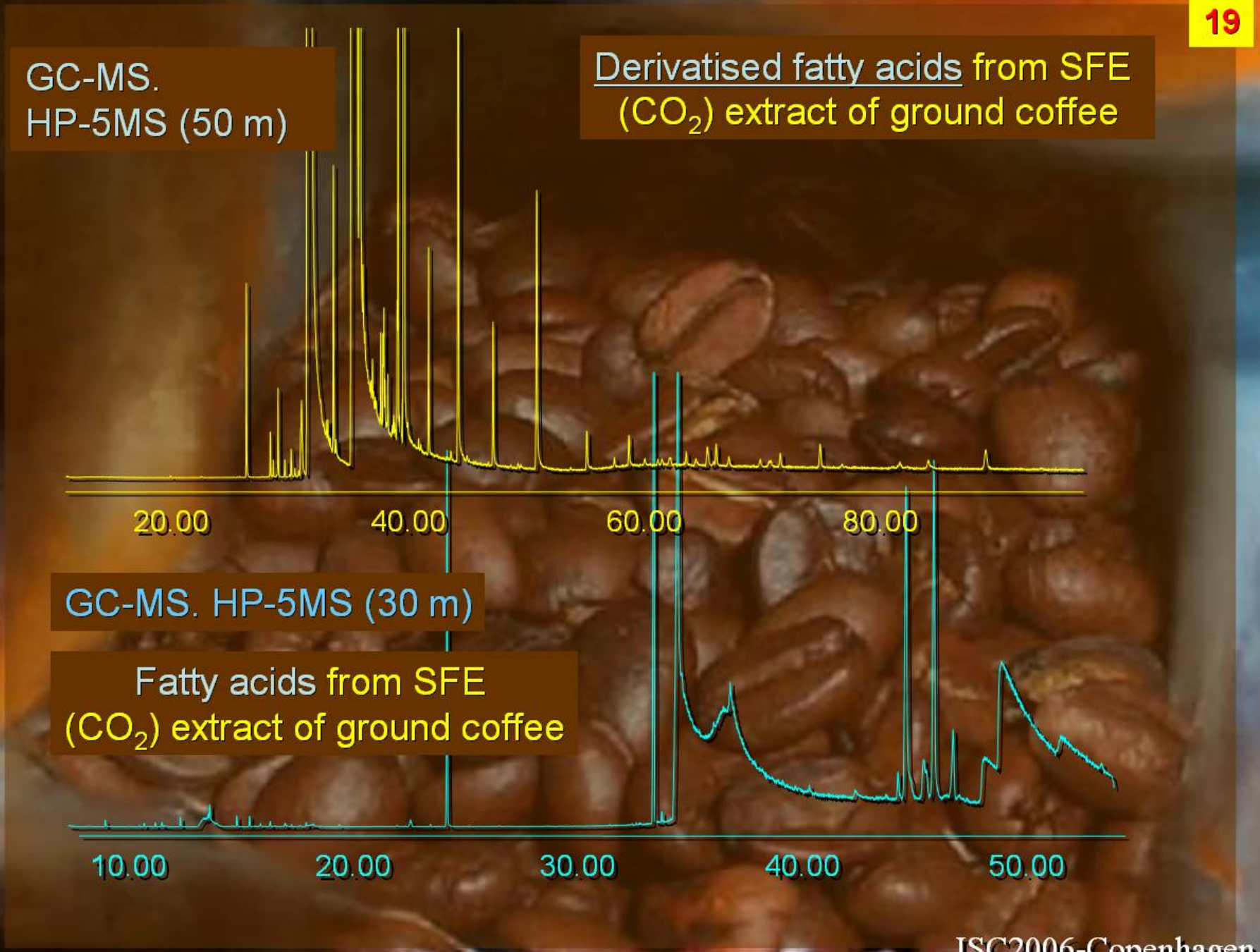


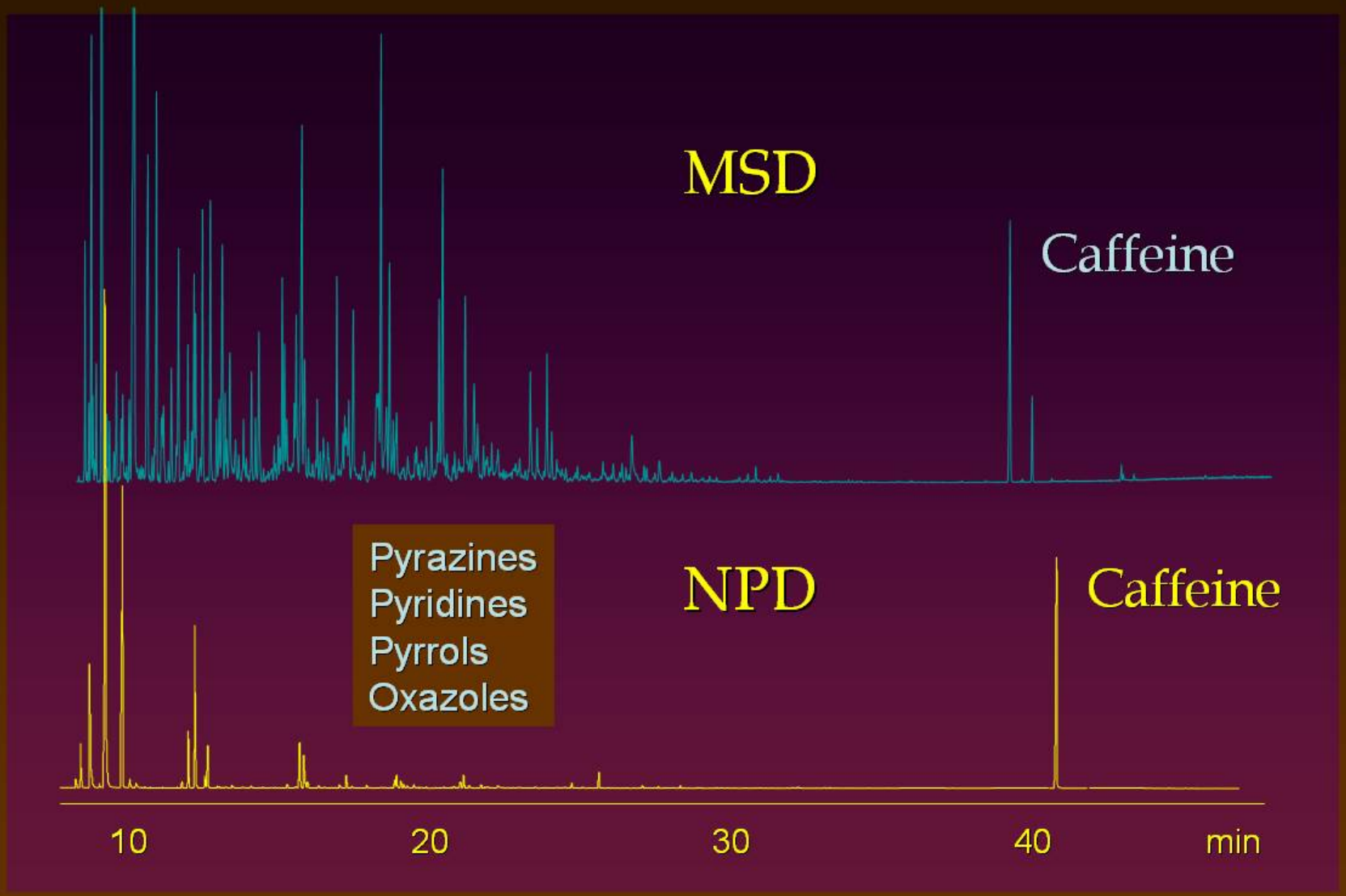
HS 100°C (2 h)



HS 70°C (2 h)

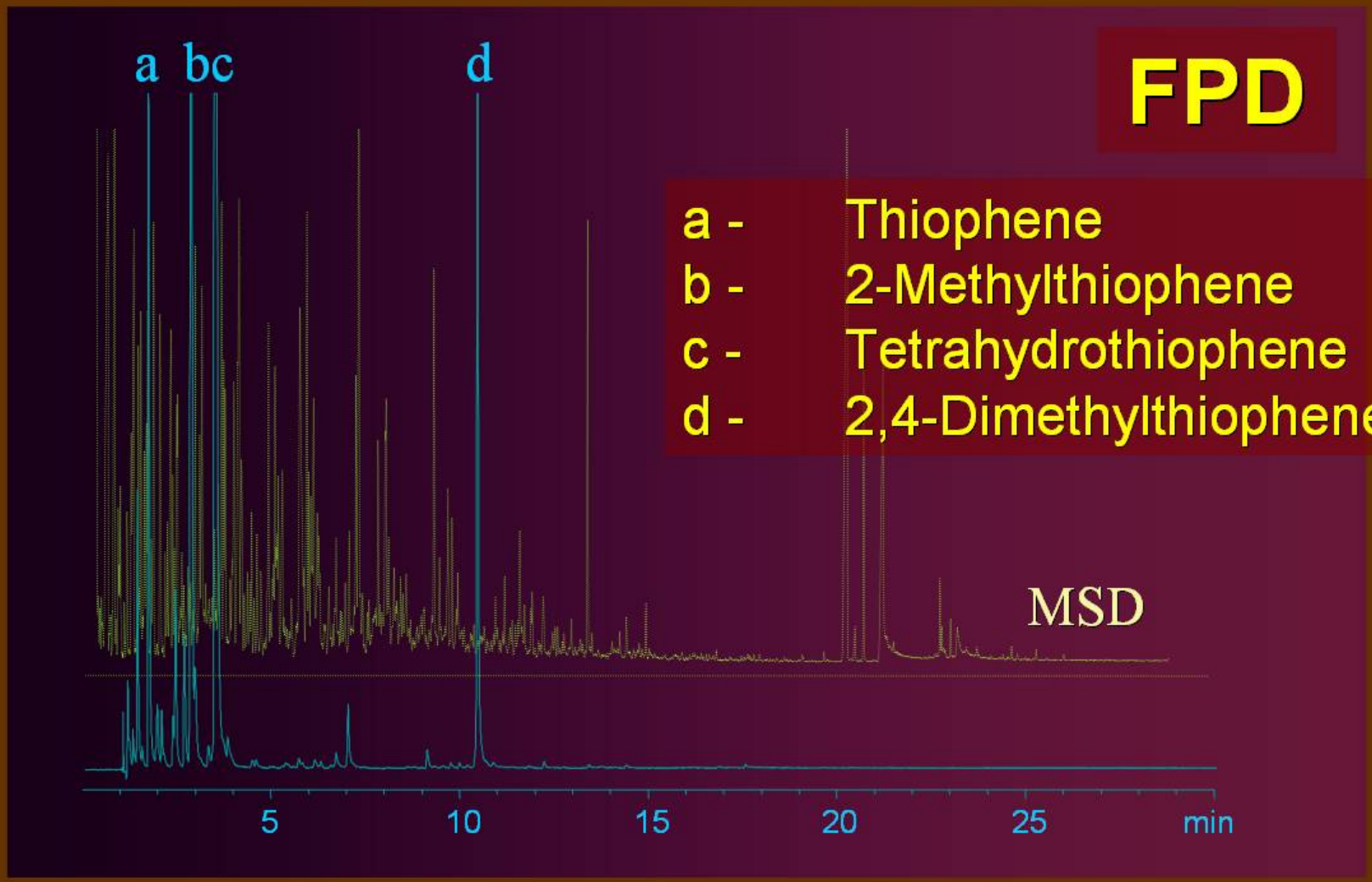
Elena Stashenko, *J. Agric. Food Chem.*, 1992, 40(11), 2257.

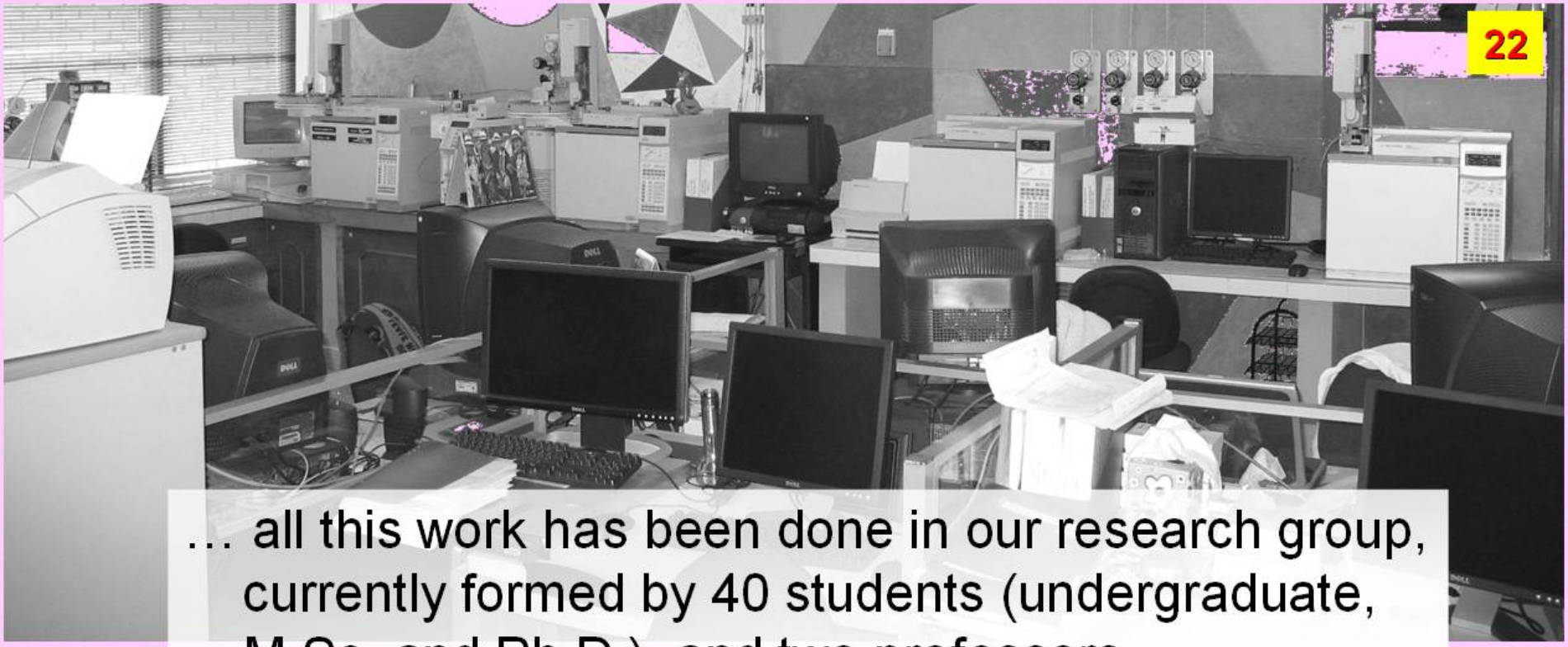

GC-MS.
HP-5MS (50 m)
Derivatised fatty acids from SFE (CO_2) extract of ground coffee
20.00
40.00
60.00
80.00
GC-MS. HP-5MS (30 m)
Fatty acids from SFE (CO_2) extract of ground coffee
10.00
20.00
30.00
40.00
50.00



Typical chromatograms of ground coffee extracts obtained by simultaneous purging (HS) - solvent extraction. HP-5 column (50 m x 0.25 mm x 0.33 µm).

Heterocyclic compounds: Sulphur compounds



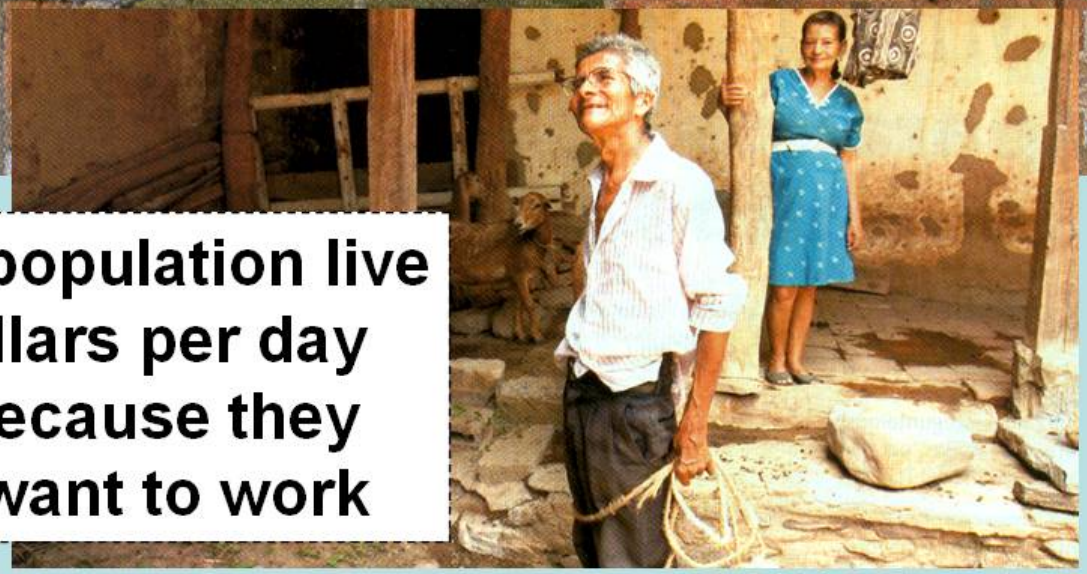


… all this work has been done in our research group, currently formed by 40 students (undergraduate, M.Sc. and Ph.D.), and two professors.

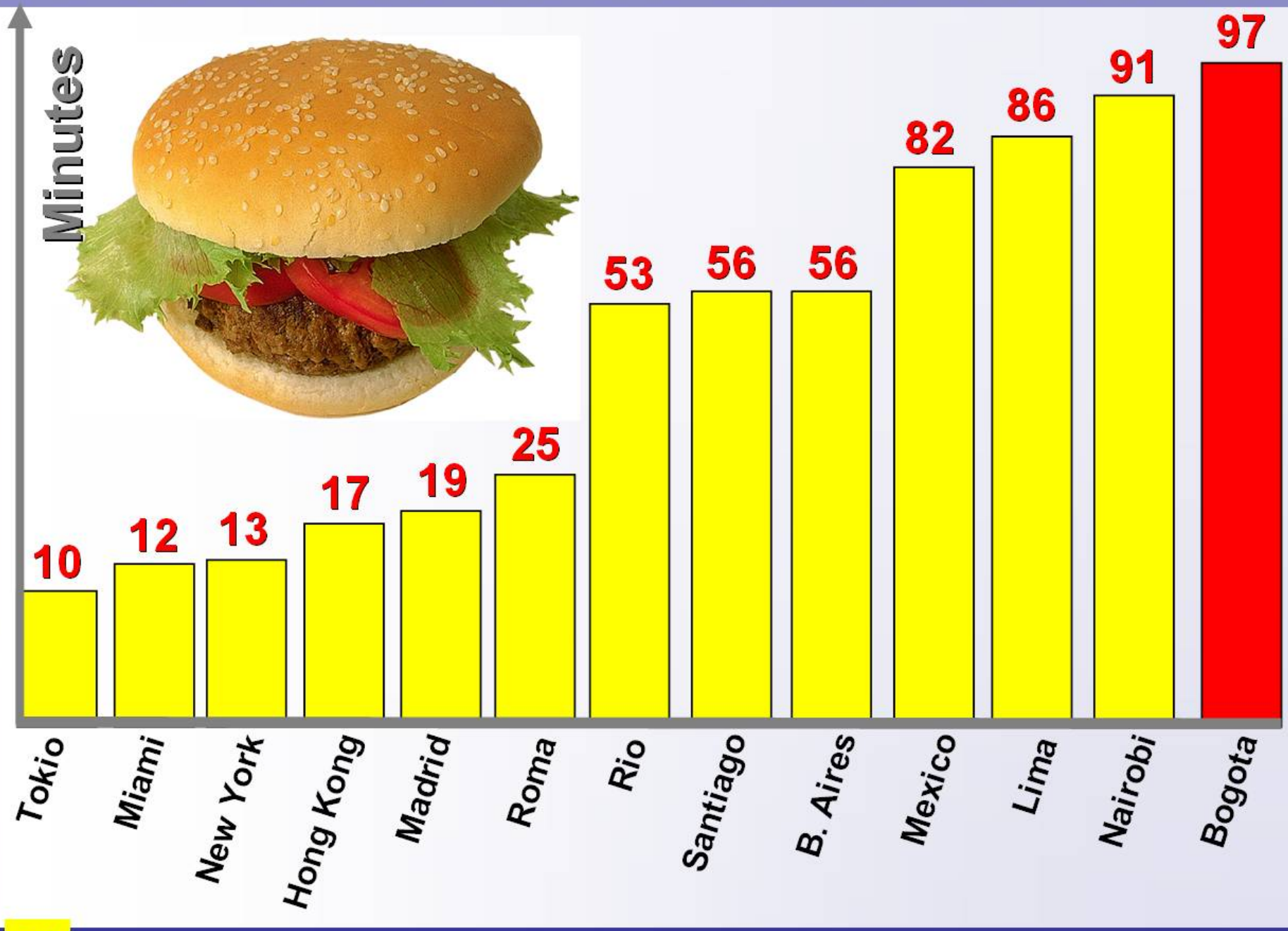
but…

… demands attention to many other aspects

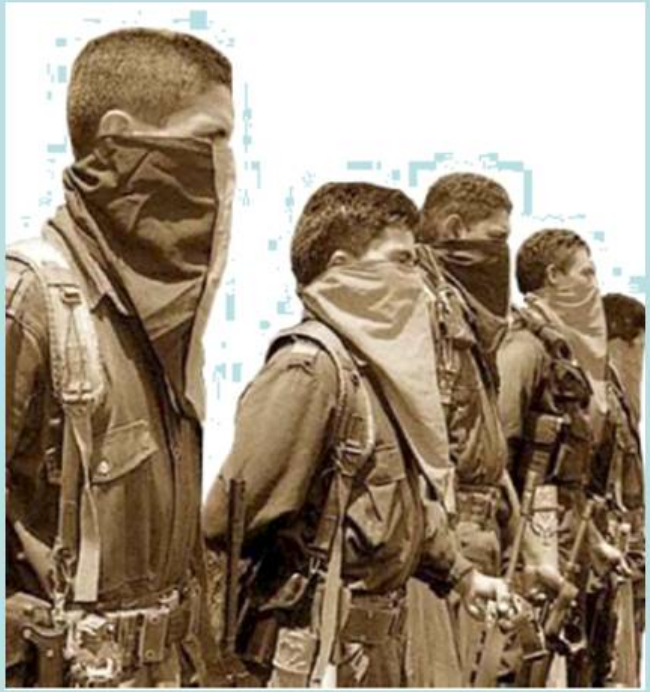
Poverty



More than 60% of the population live with less than 1.5 dollars per day … and not exactly because they are lazy or DO NOT want to work


Minutes
10
12
13
17
19
25
53
56
56
82
86
91
97
Tokio
Miami
New York
Hong Kong
Madrid
Roma
Rio
Santiago
B. Aires
Mexico
Lima
Nairobi
Bogota

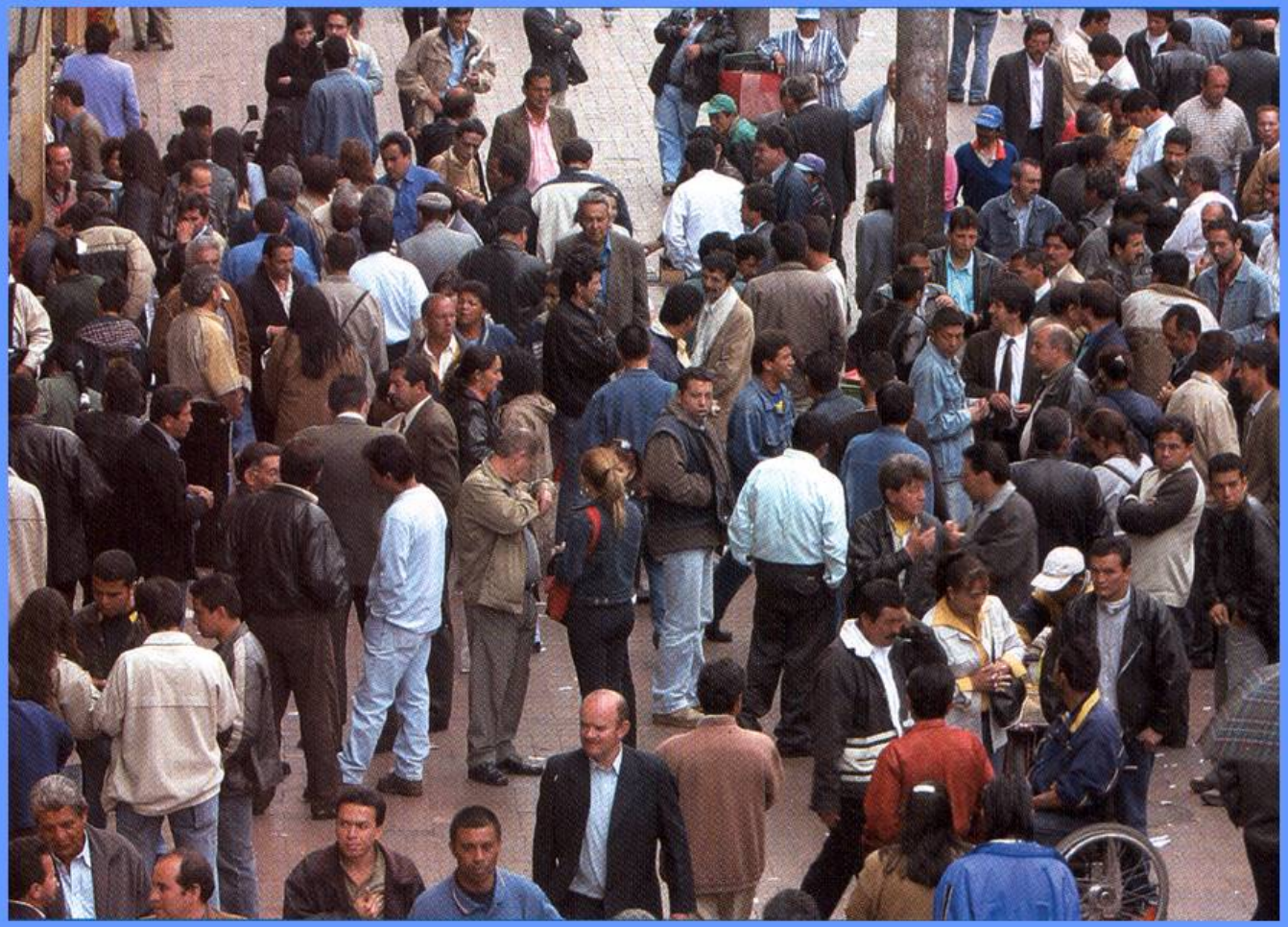
- Colombia is one of the countries with the largest number of refugees
- First place in kidnapping



WAR (on drugs?): Colombia receives the second (after Israel) largest military aid from U.S.

Paramilitaries are responsible for a huge majority of the massacres in Colombia

Areas of Guerrilla and Paramilitary Activity



Unemployment (>20% ?)

Narco-traffic

Narco-traffic:

- Death
- Violence
- Corruption
- Contamination
- Deforestation

Glyphosate spray (fumigation)

- Iliteracy
- Technological dependence
- Inequality
- Wealth concentration
- Extinction of the Middle Class
- Idiosyncrasy

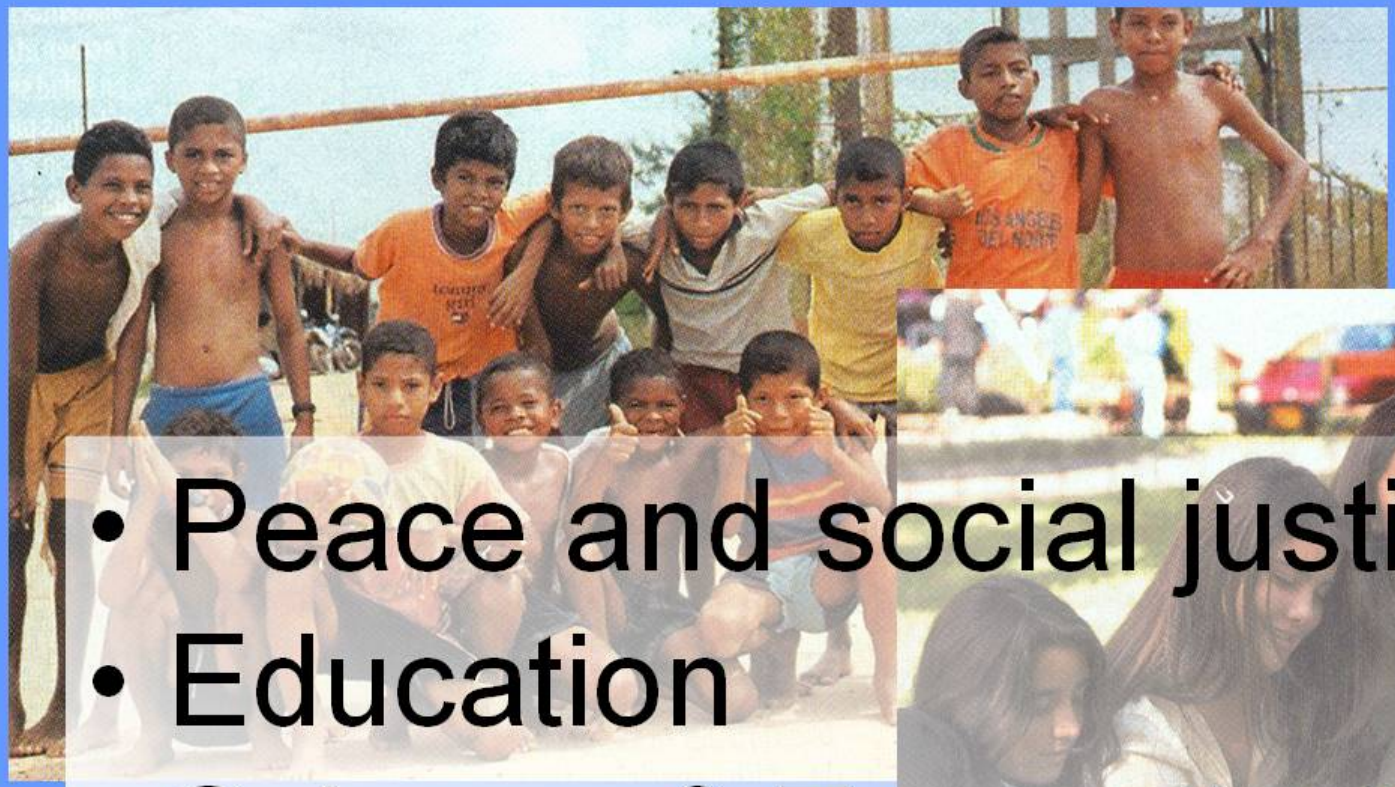
•Brain drain

Colombia spends in Science & Technology in one year, the amount that the U.S. spends in one day.

- Cost of scientific research
- Technological Dependence

US $ 250 - 300
Vs
US $ 25 - 30
per sample

Solutions, ways out… ?



- Peace and social justice
- Education
- Science & Technology
- **Projects with economic and social impact**

- People
- Equipment
- *Know-how*
- Funds

… + and tenacious desire to go forward, without weakening or demagogy…

Beginning

- Research grants (*Colciencias*)
- **N**ational **S**chool of **C**hromatography
- Courses, Training, Consultancy
- Analytical Services (Industry)

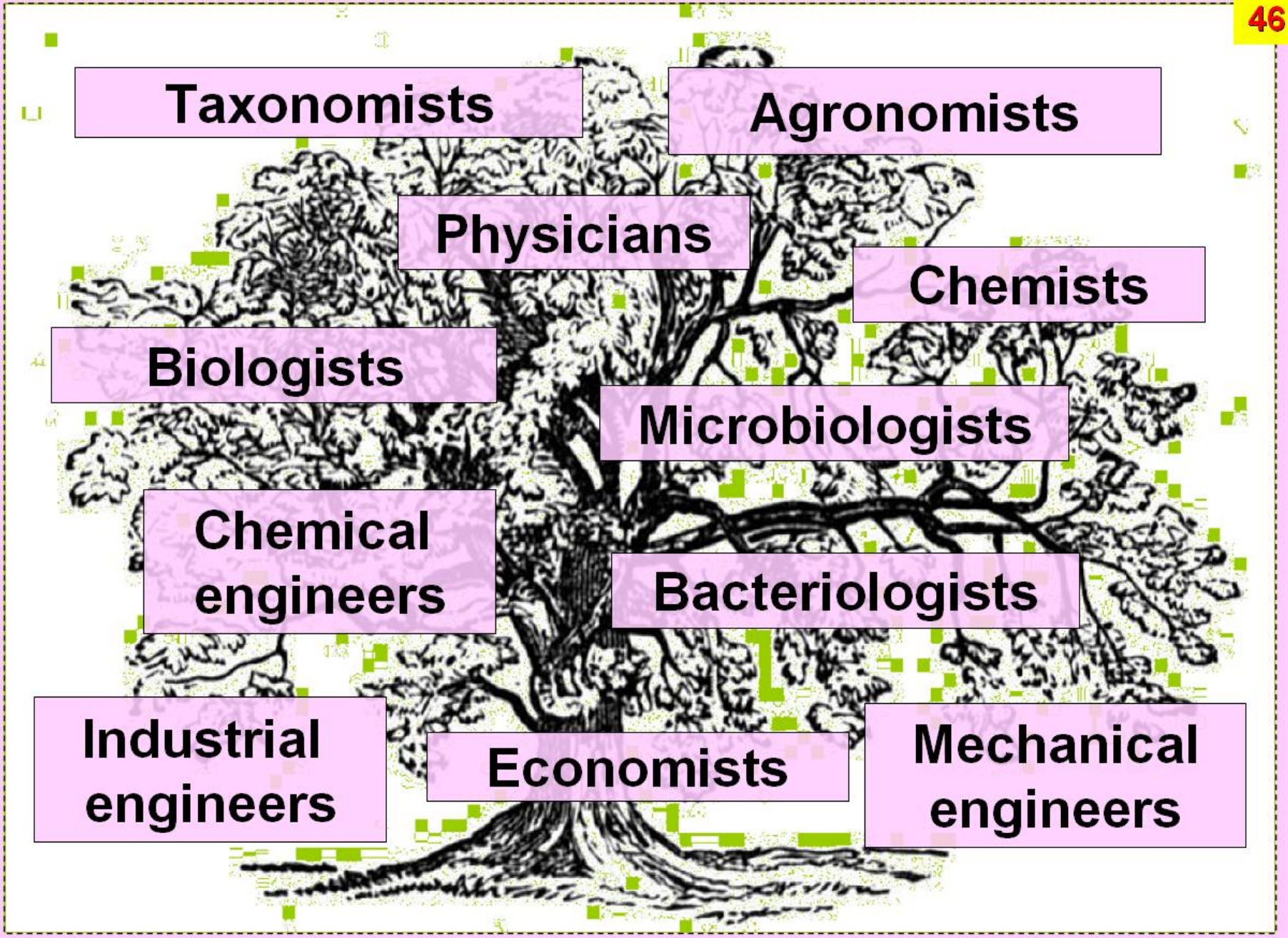
National School of Chromatography

http://cromatografia.uis.edu.co

2006

December 22, 2004, 1 p.m. Colciencias, Bogotá.
Contract signing: Research Centre of Excellence - Colciencias

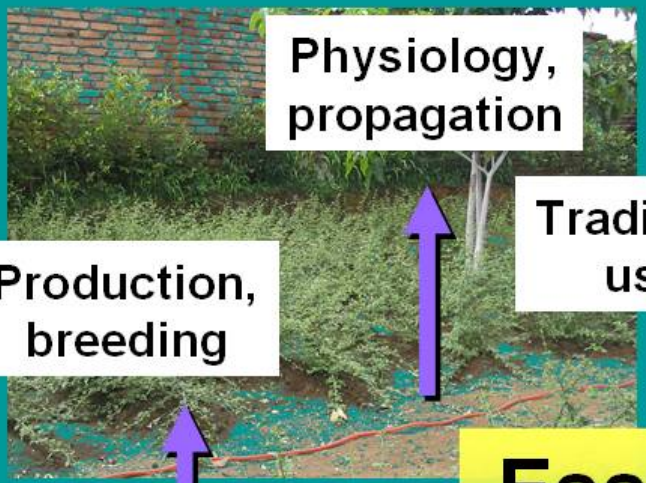
- Universidad Industrial de Santander (UIS) Bucaramanga, Santander
- Universidad de Cartagena, Cartagena, Bolivar
- Universidad de Antioquia, Medellín, Antioquia
- Universidad Tecnológica del Chocó, Quibdó, Chocó
- Universidad Tecnológica de Pereira, Pereira, Risaralda
- Fundacion Colombiana para la Farmacia Natural (Fundacofan) Cali, Valle del Cauca


Taxonomists
Agronomists
Physicians
Chemists
Biologists
Microbiologists
Chemical engineers
Bacteriologists
Industrial engineers
Economists
Mechanical engineers

Economic and social alternatives: Coca plantations substitution

Research Centre: 5 main steps of the integral study under way:

1. Ethno-botany and taxonomy;
2. Extraction and characterisation of essential oils and extracts;
3. Evaluation of extracts and essential oils bioactivity;
4. Synthesis, derivatisation and catalytic transformation of essential oils and bioactive compounds isolated from plants;
5. Technology transfer, cultivation and propagation of promising species, pilot plants for essential oil extraction, extracts isolation, and hemi-synthesis.



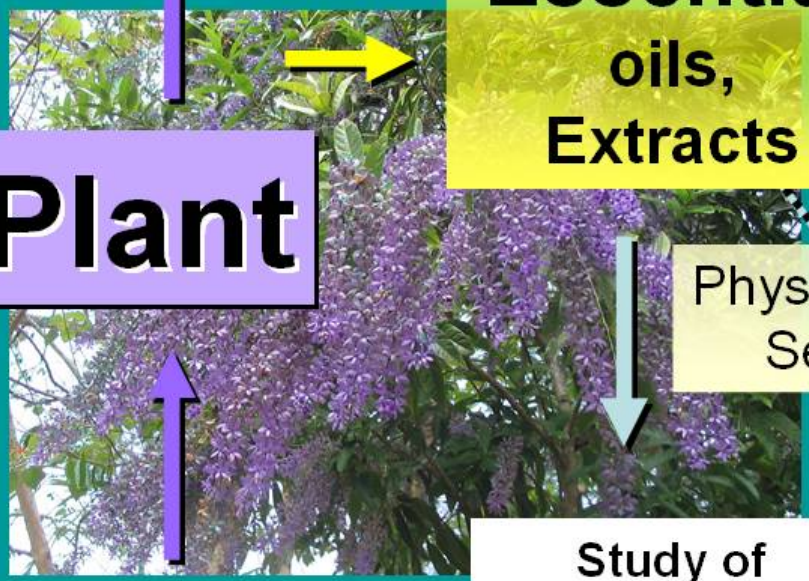
Physiology, propagation

Traditional uses

Production, breeding

New uses

- Market
- Feasibility study
- Business plan
- *Marketing*
- *Technology transfer*



Plant

Essential oils, Extracts

Essential oils, important sources of substances of high value

Physico-chemical Separacion

Transformations:

- Chemical
- Catalytic
- Enzimatic

Study of biologic activity

Search, taxonomy

Botanical expeditions: Plant collection

CODIGO 004
N.C. Astronium graveolens
ANACARDIACEAS
FAM.
N.V. Tibiyaro, Sibiyaro
Resina usada como labrus de
Heridas ectoparásitos del ganado y
Contra hongos de las uñas del
ganado
COD 016
N. Machaerium
FAM FABACEAE
TRIBU DALBERGIEE TIENE SIMBIO-
LA TRIBU DALBERGIEE TIENE SIMBIO-
CAS TENCIAS INSECTICIDAS Y FUNGICI-

- Reaching out to the community
- Field work
- Training workshops
- Technical assistance, instruction

Simití, South Bolivar,
April, 2005

July, 2005
San Pablo, South Bolivar

Cantagallo, South Bolívar, December, 2005

LABORATORIO DE PAZ
NOMBRE DE LA OBRA
CONSTRUCCION DEL CENTRO DE ACOPIO MORELIA EN EL MUNICIPIO DE BOLIVAR - SANTANDER
LA UNION EUROPEA APORTA A LA FINANCIACION DE ESTA OBRA
$65'000.000
FECHA INICIO: 20 de Septiembre de 2004
FECHA TERMINACION: 18 de Diciembre de 2004
MAGDALENA MEDIO

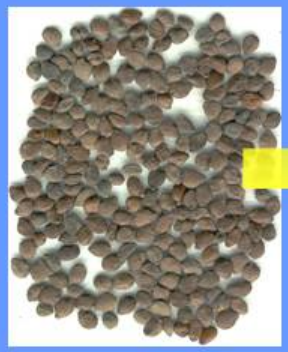
Simiti

San Blas

Monterrey

Bolívar, Santander

Productive Chain



Seeds

Seedbeds, nursery, propagation

Small plots / Genetic selection

Vegetal material treatment

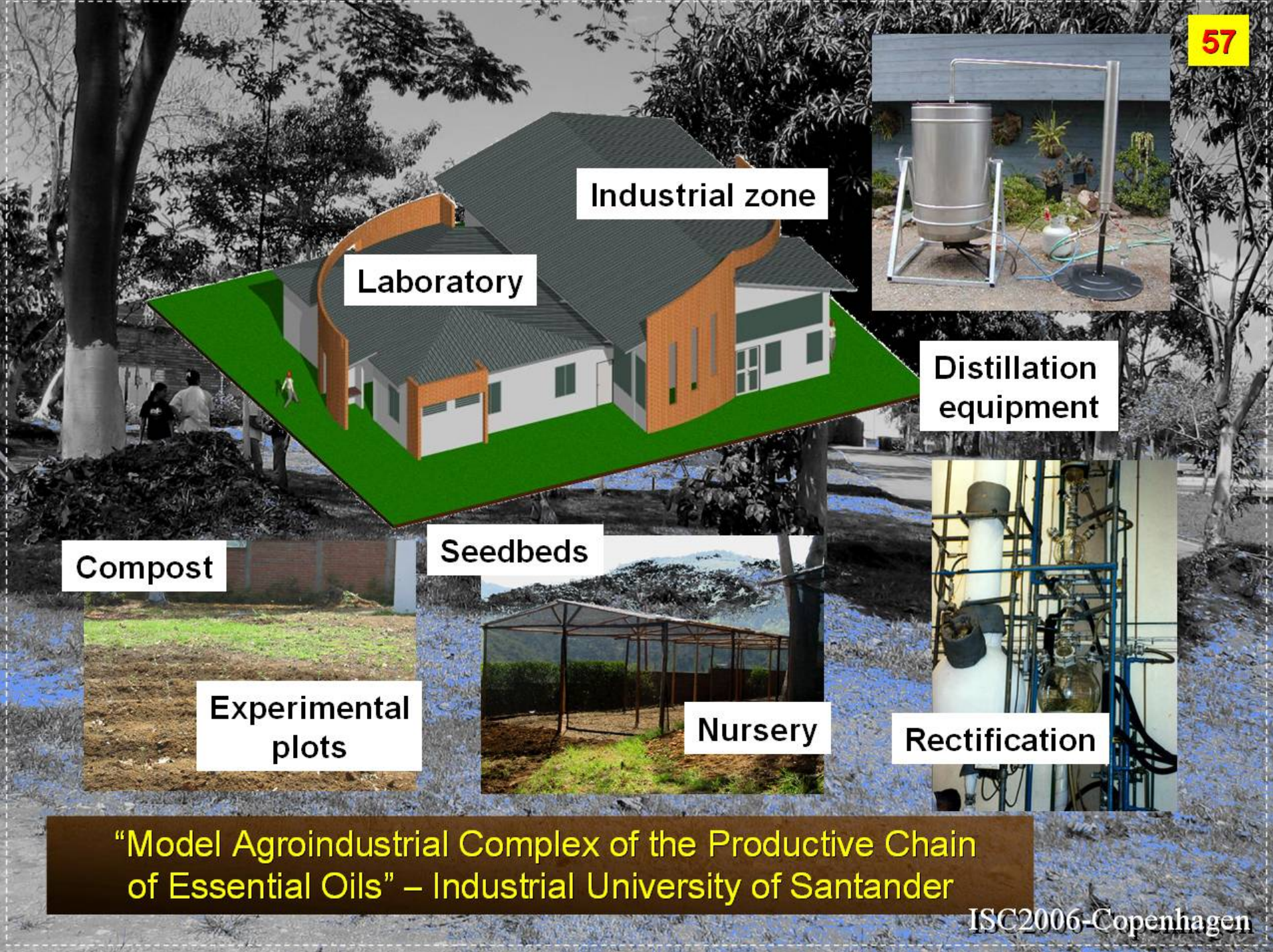
Distillation - Extraction

Compost

Quality control

Rectification

Packaging



"Model Agroindustrial Complex of the Productive Chain of Essential Oils" – Industrial University of Santander

Breaking ground…

Chemistry is the science of matter transformation

Economically promising aromatic plants

"Model Agroindustrial Complex for the Productive Chain of Essential Oils"

Development of Separation Science in Colombia

- *Technical dependence*
- *Know-how*
- *Importation, accessories and technical support*
- *Electricity*
- *Main public university*

- *Education (courses, labs)*
- *Basic investigation*
- *Applications*

Separation Science

Applications:

- Environmental
- Petrochemistry
- Food/Aroma
- Natural Products
  - Pharmaceuticals
  - Forensic
  - Doping control
- Quality control

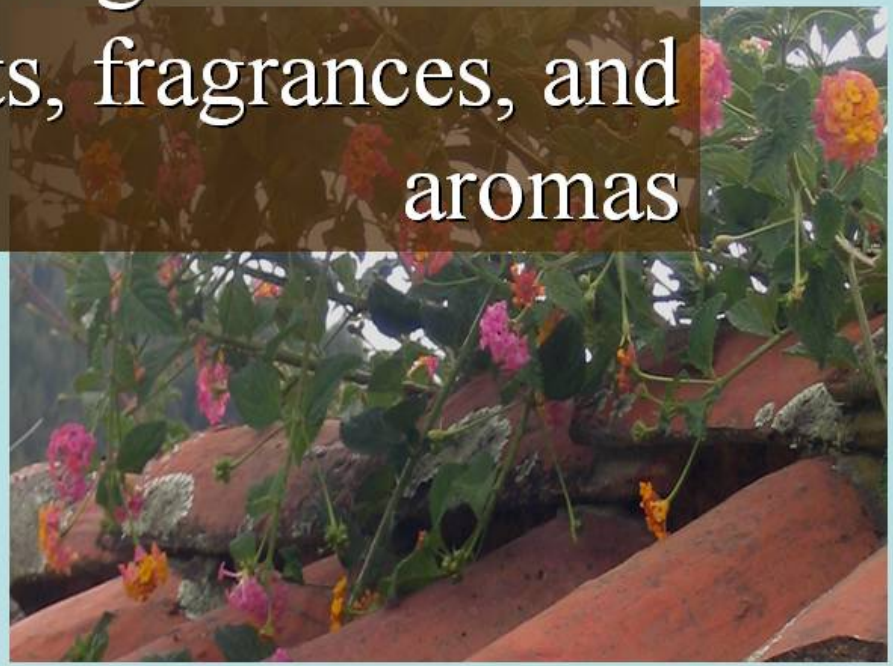
- *Universities* ◆ *Industry*
- *Government Laboratories*

Equipment:

- GC (> 300)
- HPLC (> 200)
- GC-MS (ca. 50)
- LC-MS (5)
  - CE (2)

Some examples

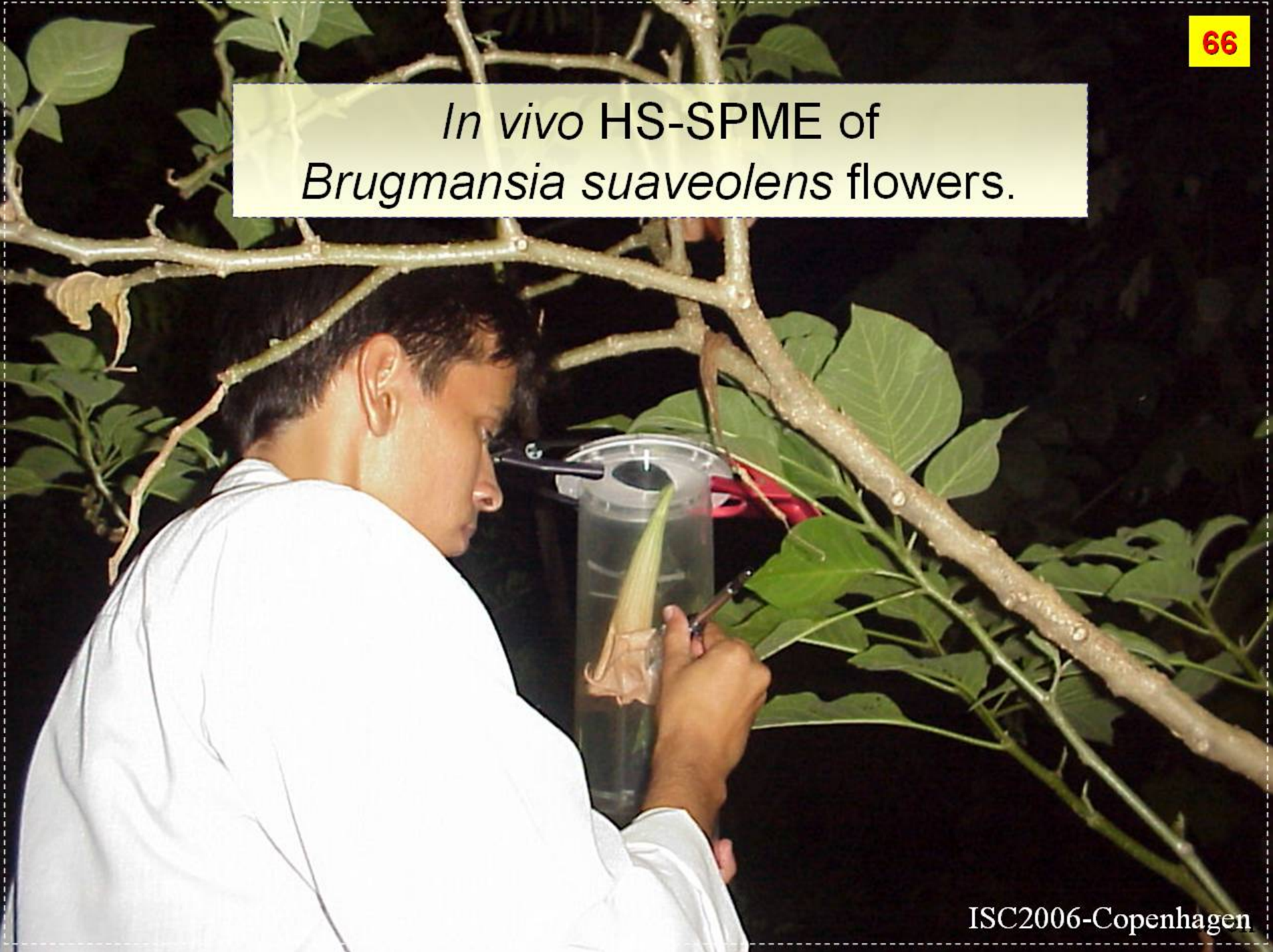
Colombia is a kingdom of exotic flowers , fruits, fragrances, and aromas

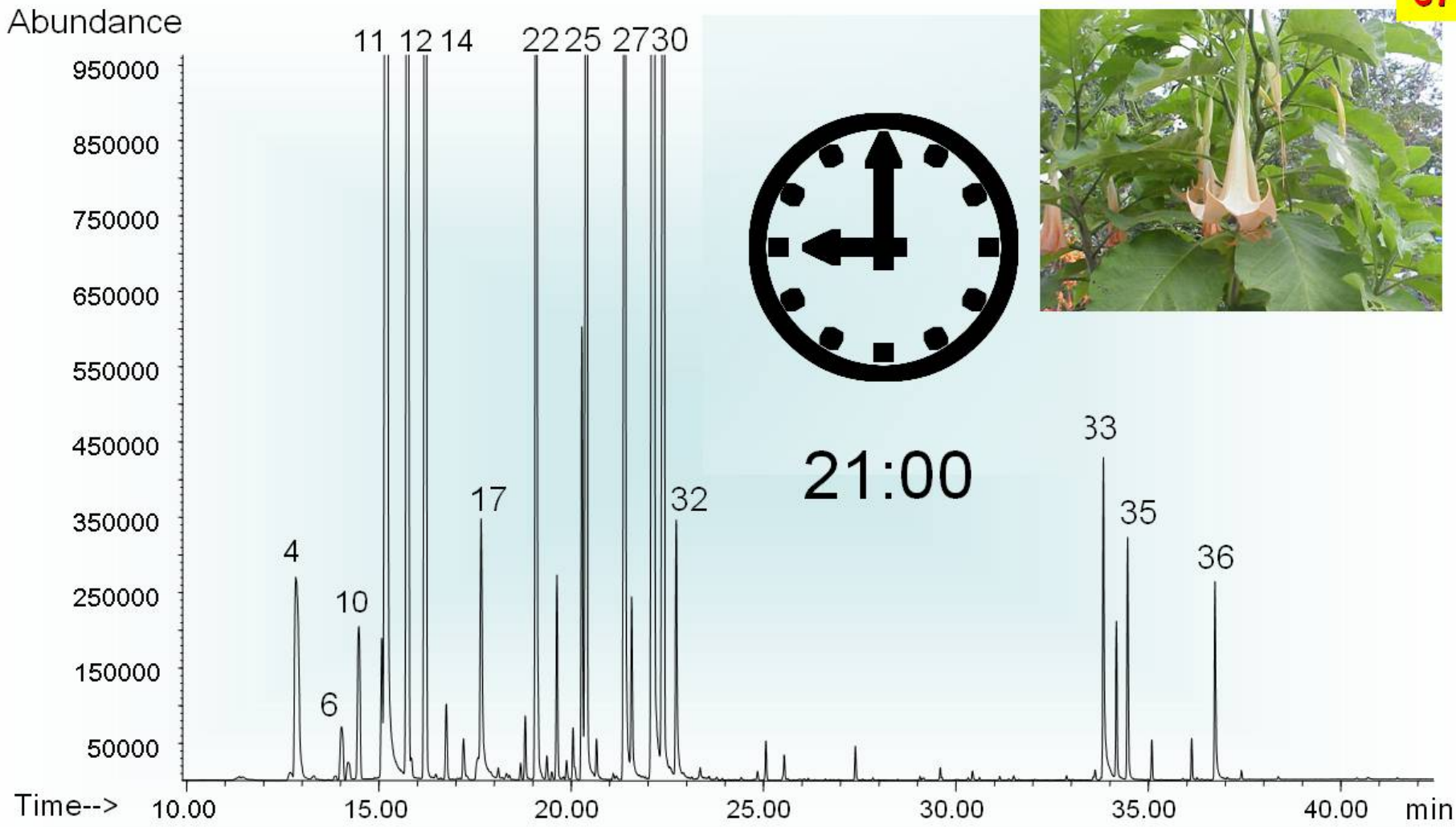


Genera:
Datura sp.
Brugmansia sp.

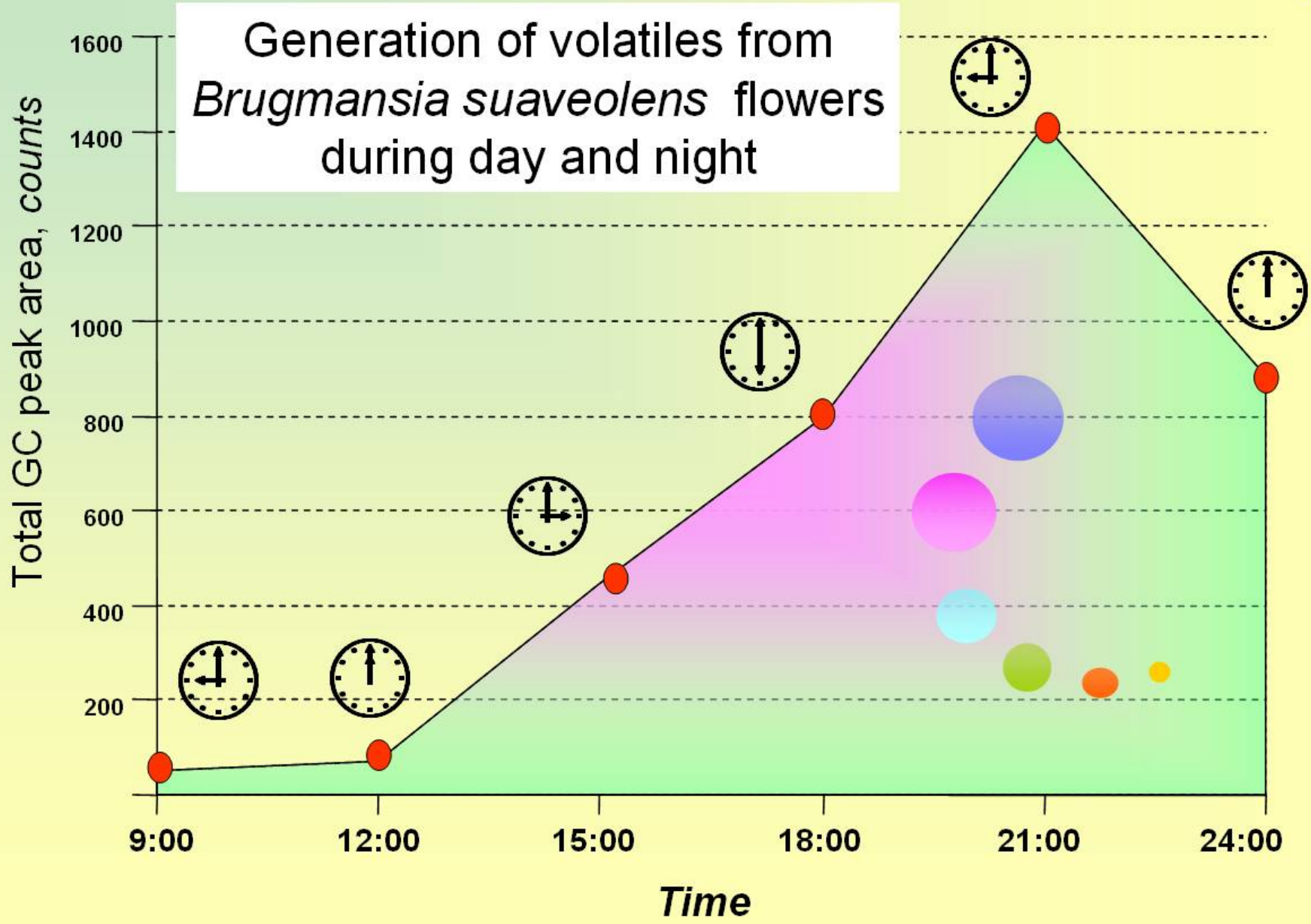
Brugmansia suaveolens

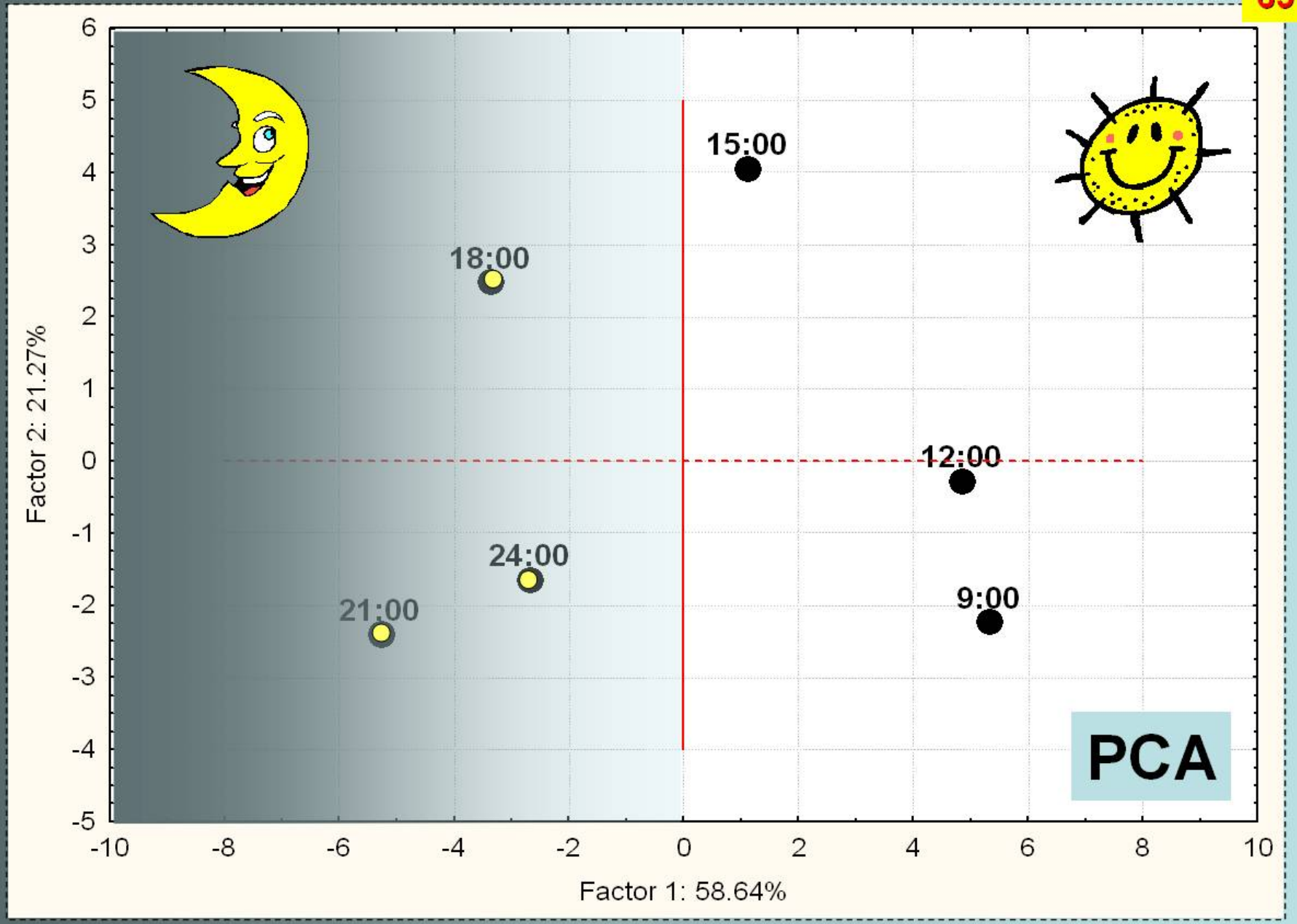
In vivo HS-SPME of *Brugmansia suaveolens* flowers.





Typical gas chromatogram of *Brugmansia suaveolens* volatiles, isolated *in vivo* by HS-SPME from flowers. DB-1 (60 m). MSD (EI, 70 eV).

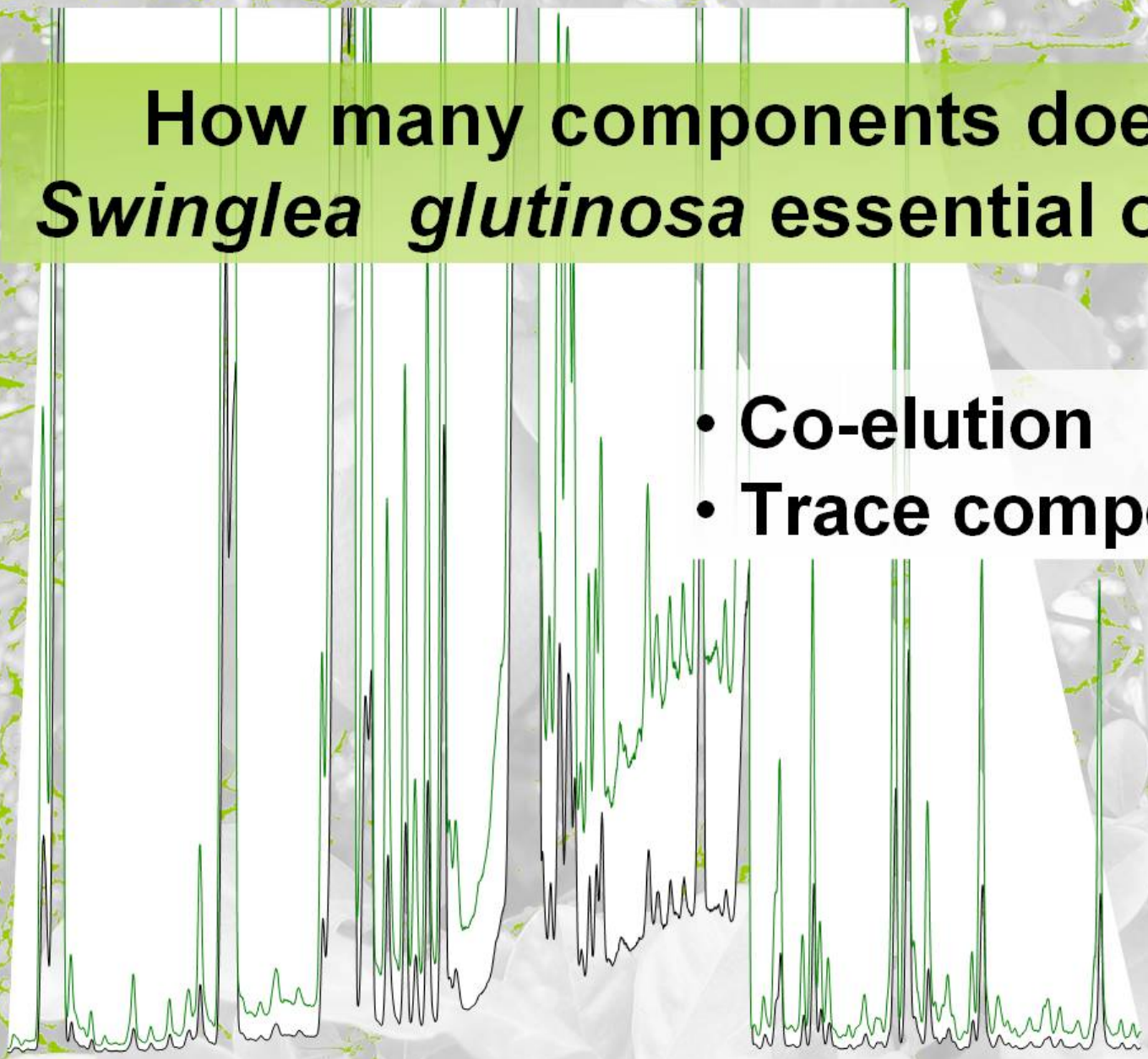

Generation of volatiles from
Brugmansia suaveolens flowers
during day and night
Total GC peak area, *counts*
1600
1400
1200
1000
800
600
400
200
9:00
12:00
15:00
18:00
21:00
24:00
Time


15:00
18:00
12:00
24:00
9:00
21:00
PCA
Factor 2: 21.27%
Factor 1: 58.64%
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-10
-8
-6
-4
-2
0
2
4
6
8
10

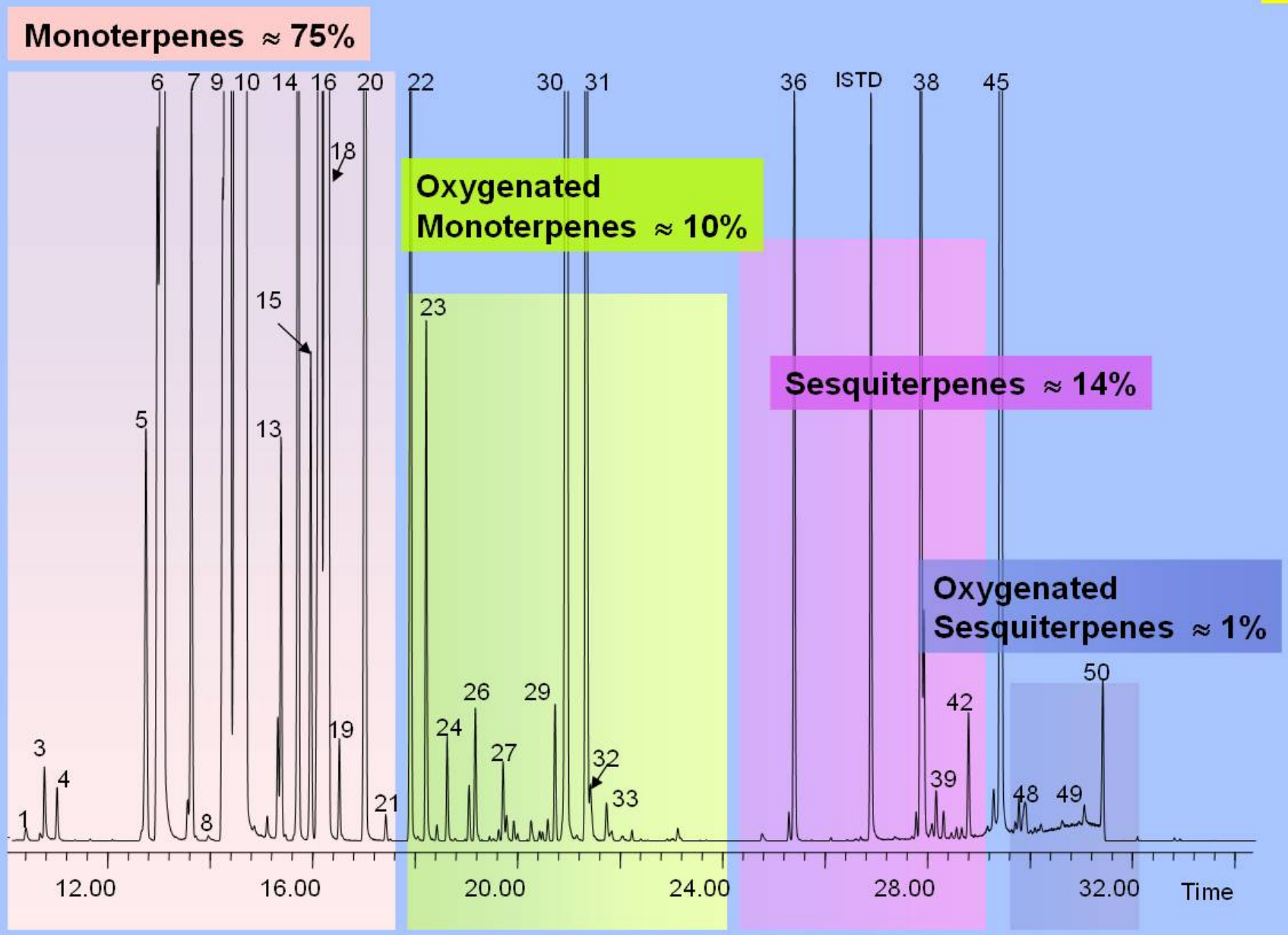
Swinglea glutinosa is a small tropical tree from the *Rutaceae* family.

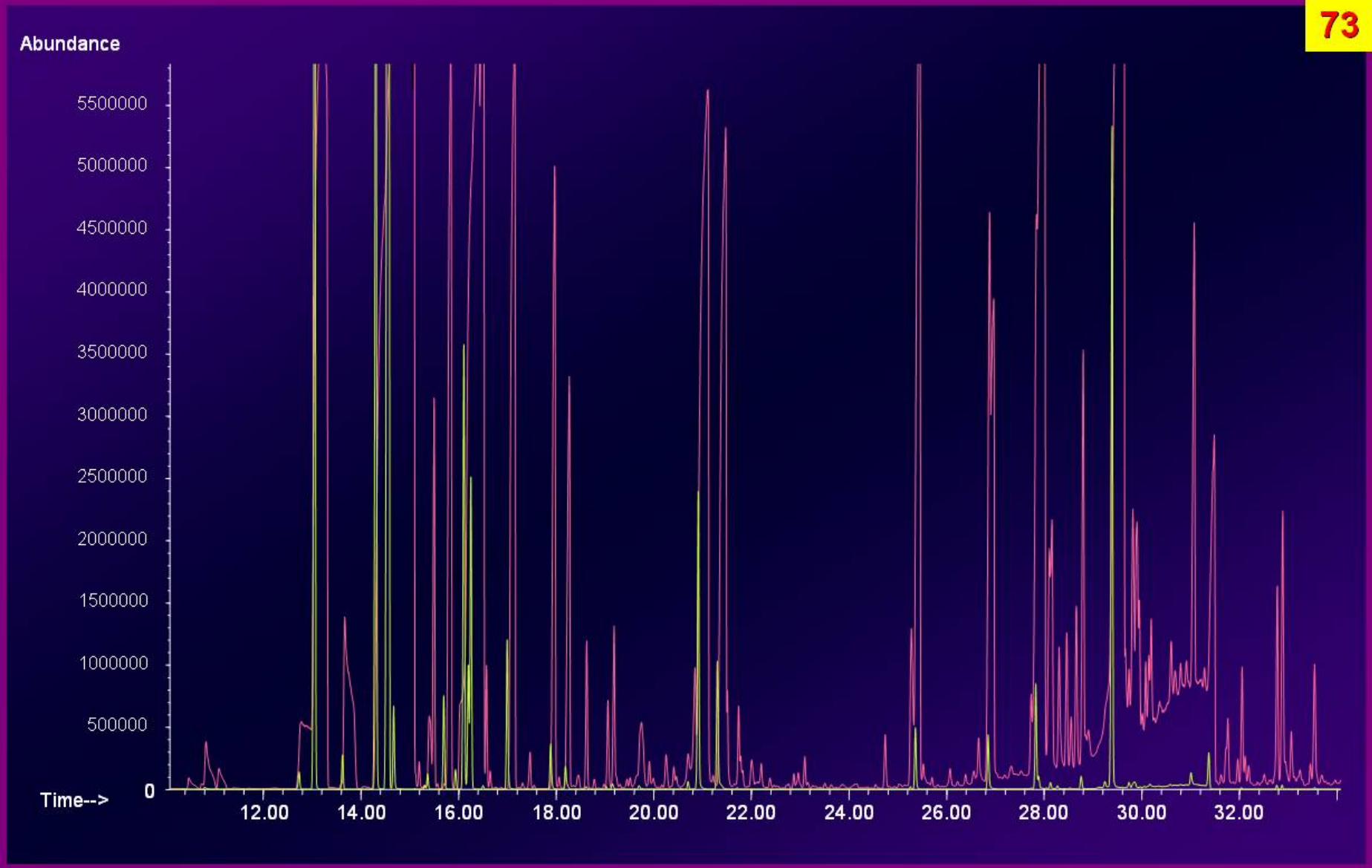
Lemon
Lime
Mandarin
Orange

How many components does the *Swinglea glutinosa* essential oil have?



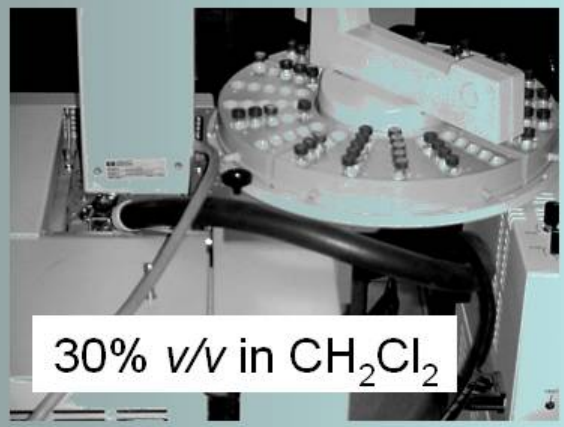
- Co-elution
- Trace components


Monoterpenes ≈ 75%
Oxygenated Monoterpenes ≈ 10%
Sesquiterpenes ≈ 14%
Oxygenated Sesquiterpenes ≈ 1%
6 7 9 10 14 16 20 22 30 31 36 ISTD 38 45
18
15
23
5
13
50
42
26 29
24
19
27
32
3
4
39
48 49
33
1
8
21
12.00
16.00
20.00
24.00
28.00
32.00
Time

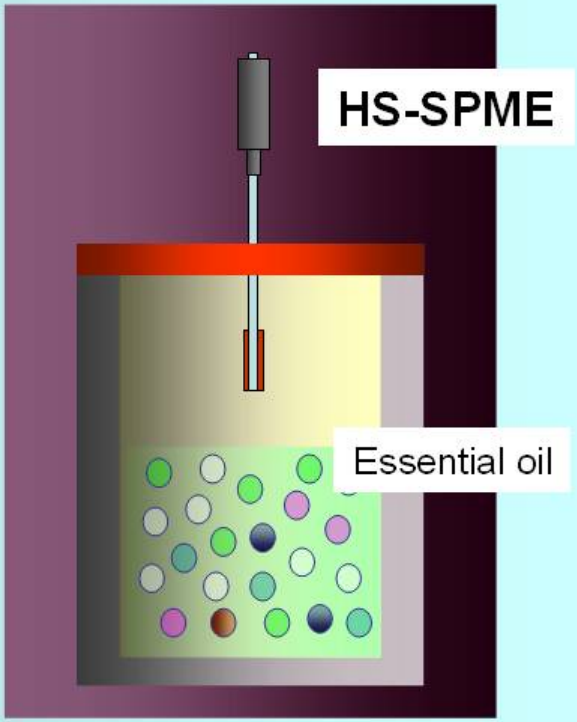


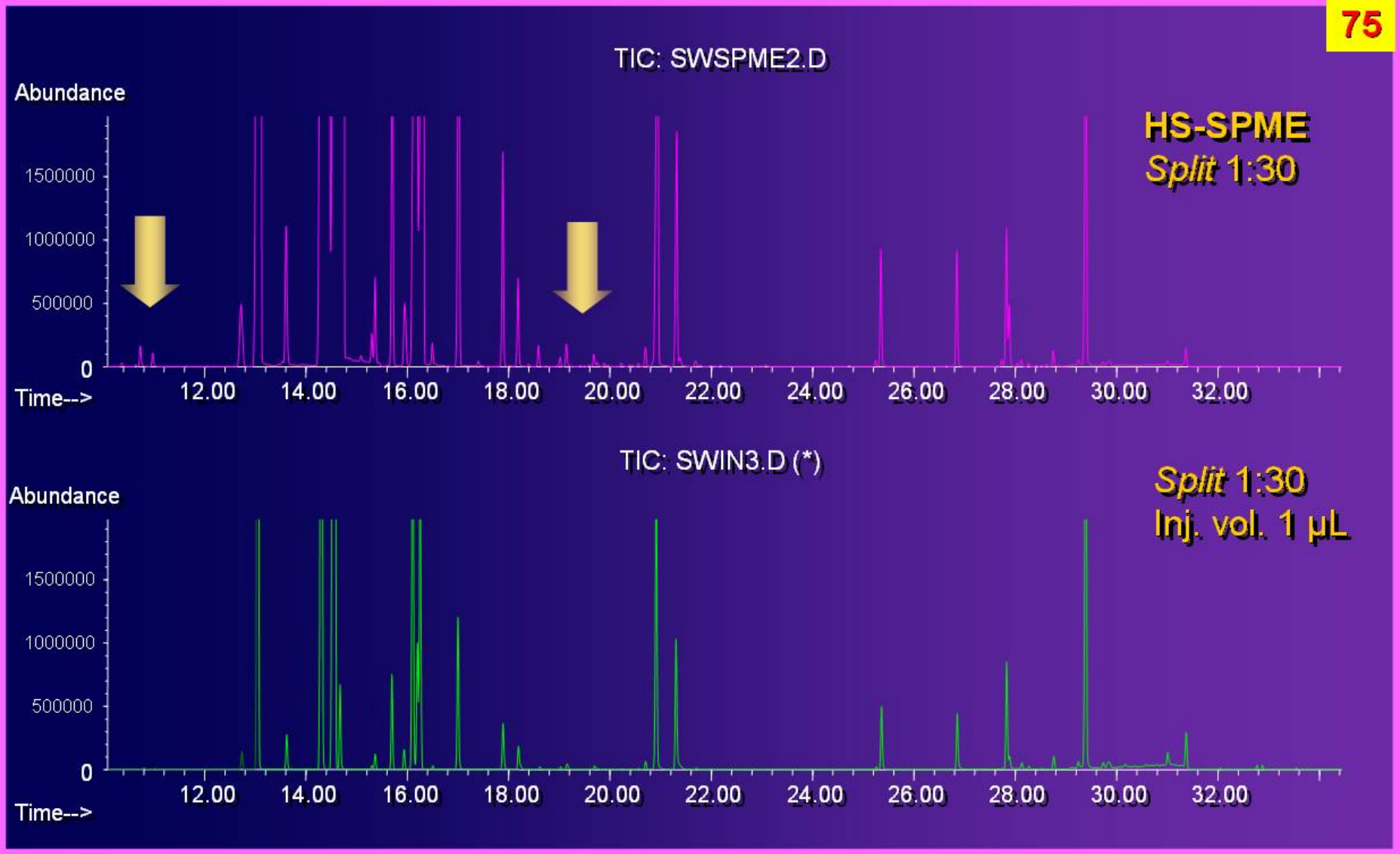
Split 1:30 — Splitless

Inj. vol. 1 µL; DB-1*MS* (60 m)



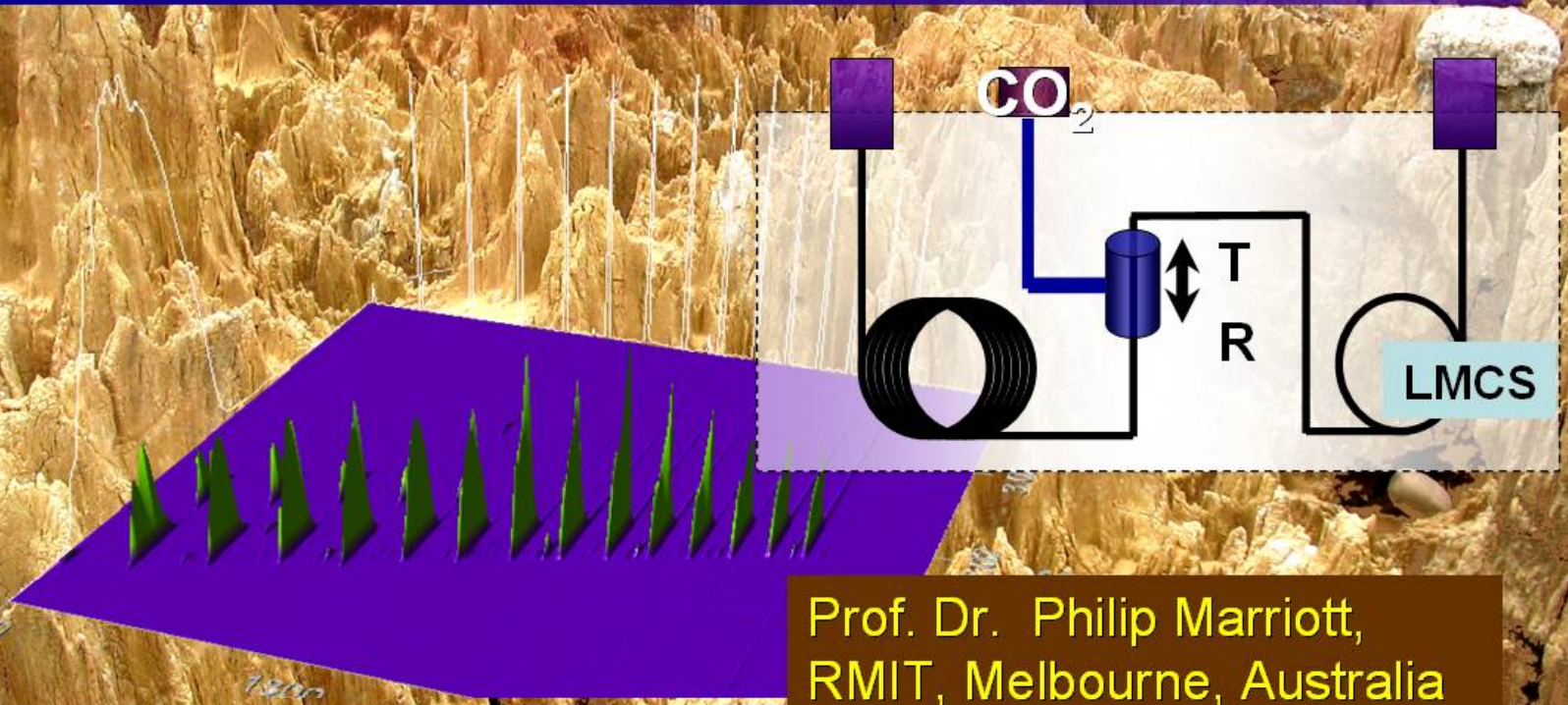
- *Split* 1:30, 1:50, 1:100
- *Splitless*
- Pulsed *splitless*
- *On-column*





MSD (EI, 70 eV). DB-1MS (60 m x 0.25 mm x 0.25 µm). *Split* 1:30.

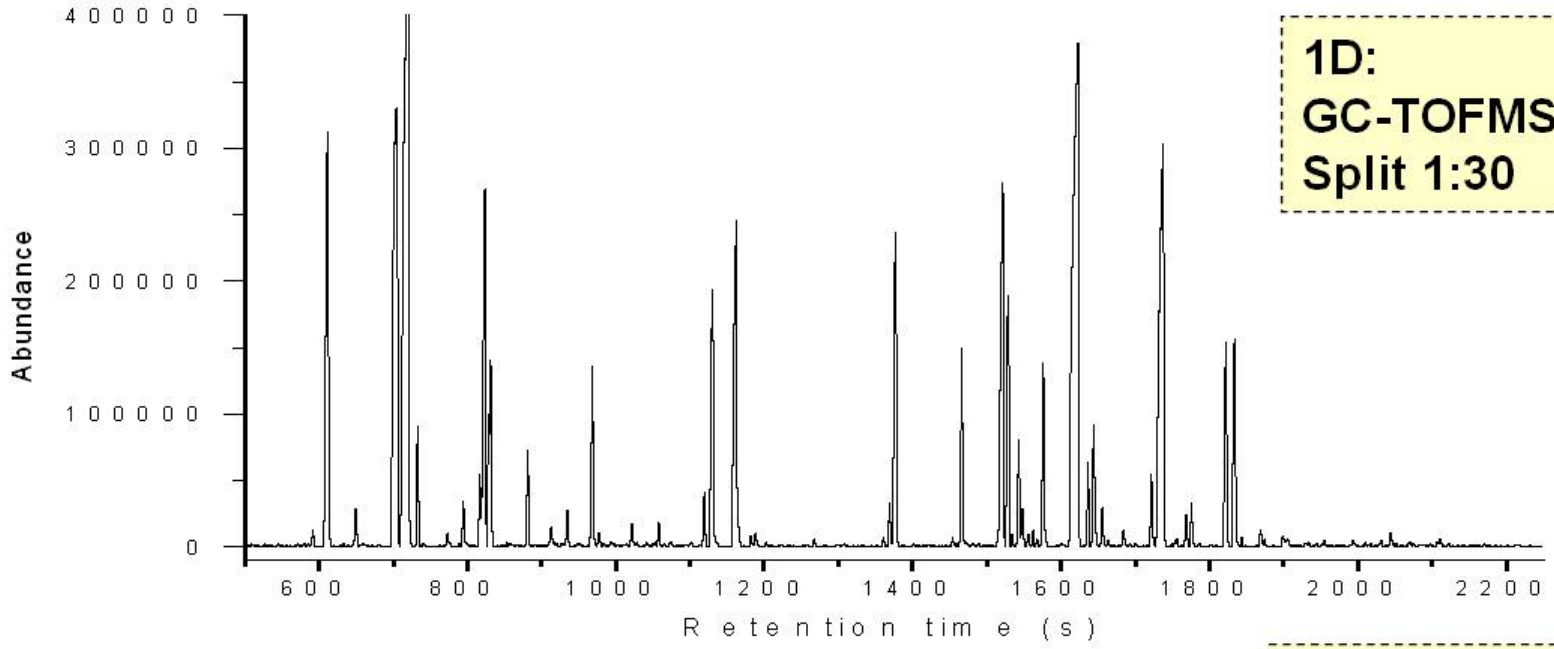
Comprehensive Two - Dimensional Gas Chromatography, GC x GC

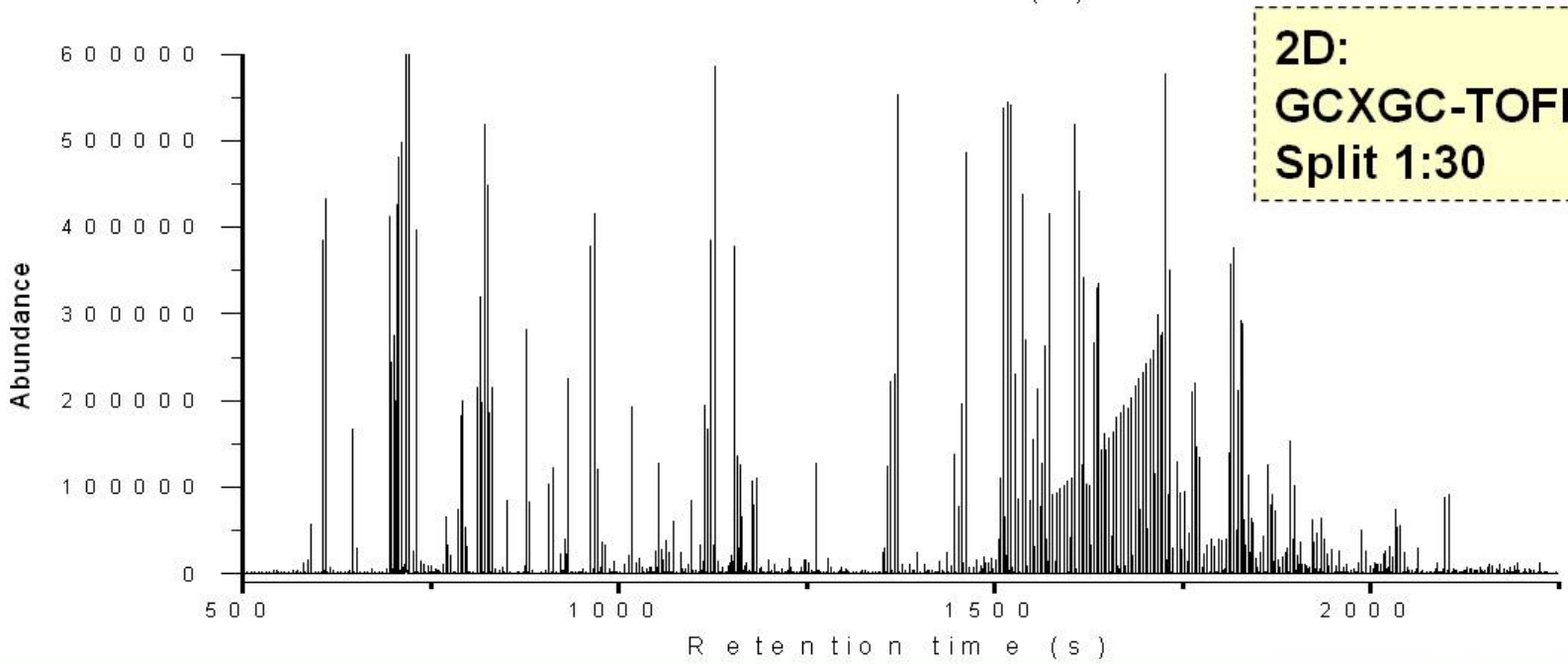


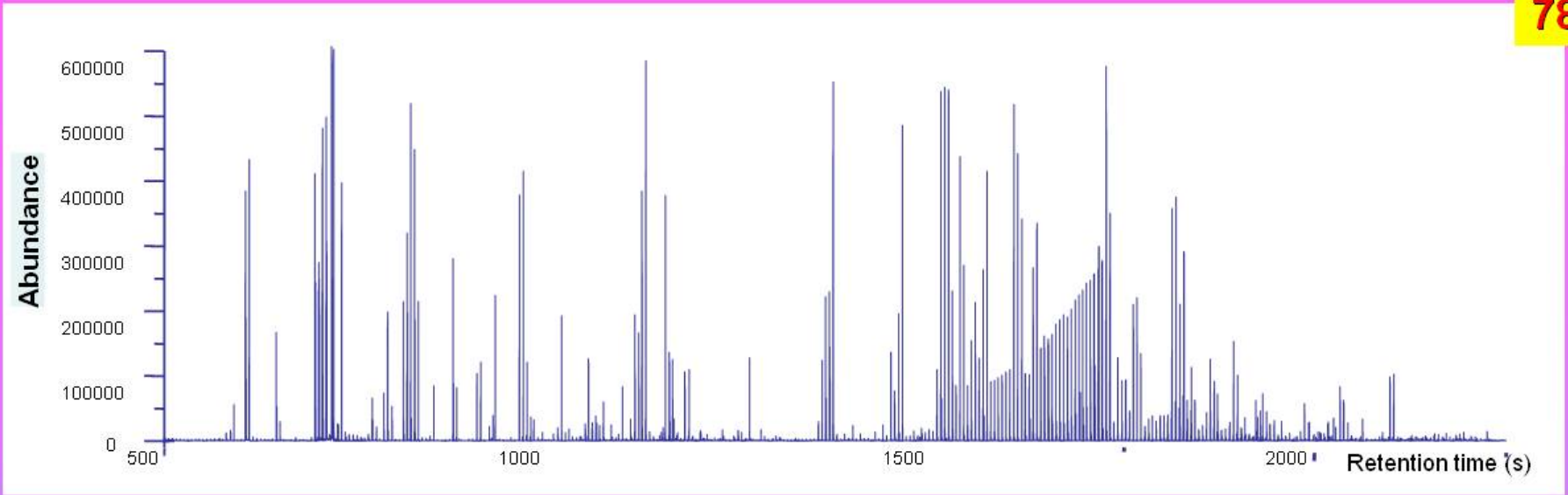
Prof. Dr. Philip Marriott,
RMIT, Melbourne, Australia

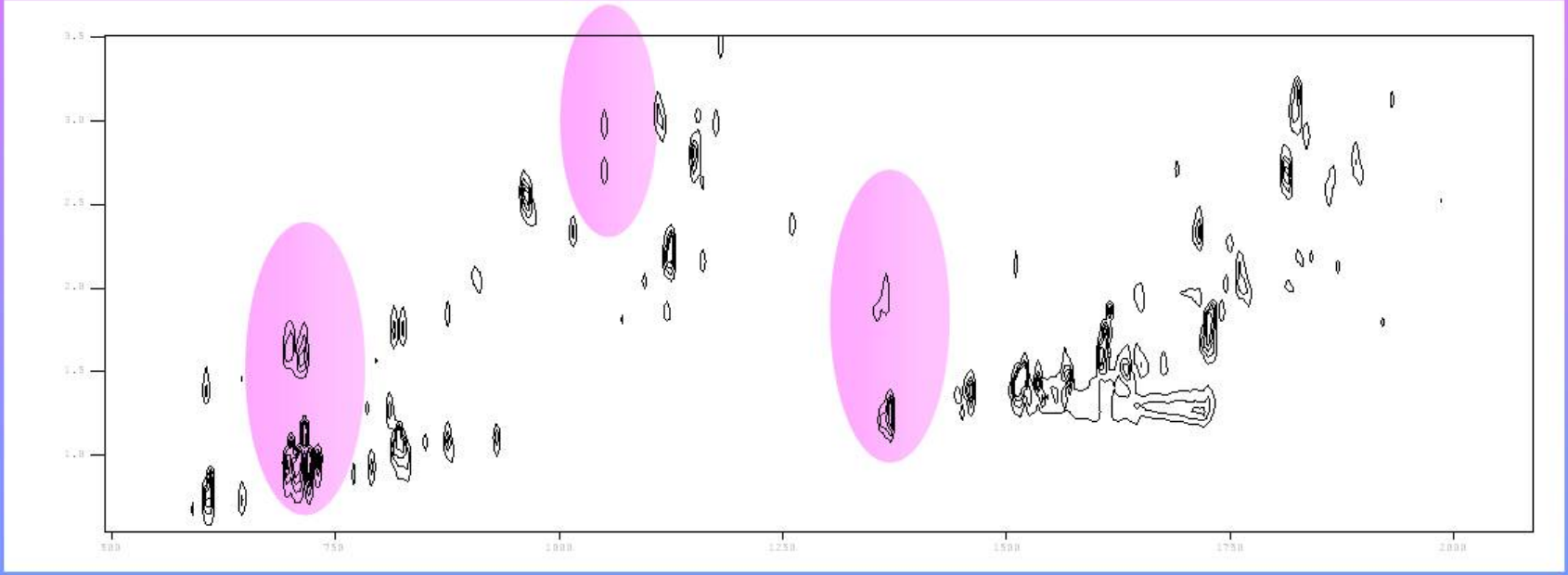
Longitudinally-modulated cryogenic system (LMCS).

R.M. Kinghom, P.J. Marriott, *J. High Resol. Chromatogr.* **1998**, *21*, 620 – 622.


1D:
GC-TOFMS
Split 1:30
Abundance
400000
300000
200000
100000
0
600
800
1000
1200
1400
1600
1800
2000
2200
Retention time (s)


2D:
GCXGC-TOFMS
Split 1:30
Abundance
600000
500000
400000
300000
200000
100000
0
500
1000
1500
2000
Retention time (s)

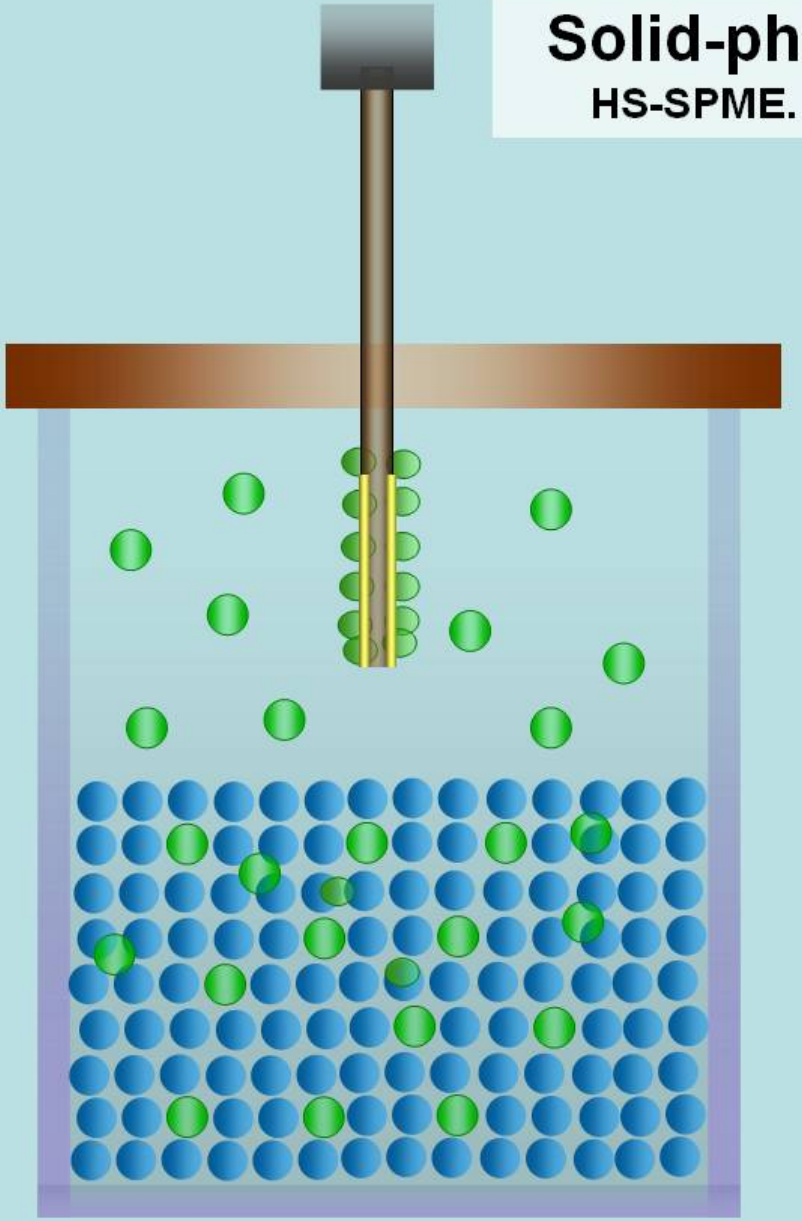


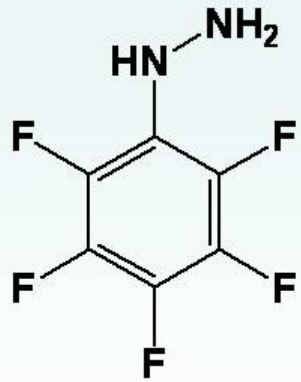


TIC (GCxGC-TOFMS) of *Swinglea glutinosa* essential oil.

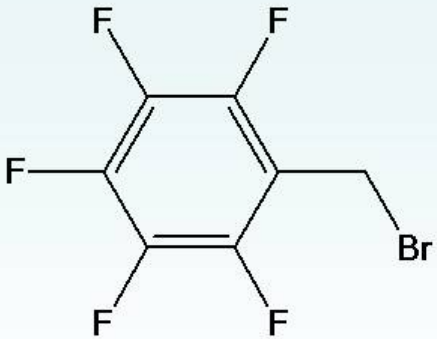
Solid-phase-based extraction methods:

HS-SPME. Fibre saturation with derivatisation reagent





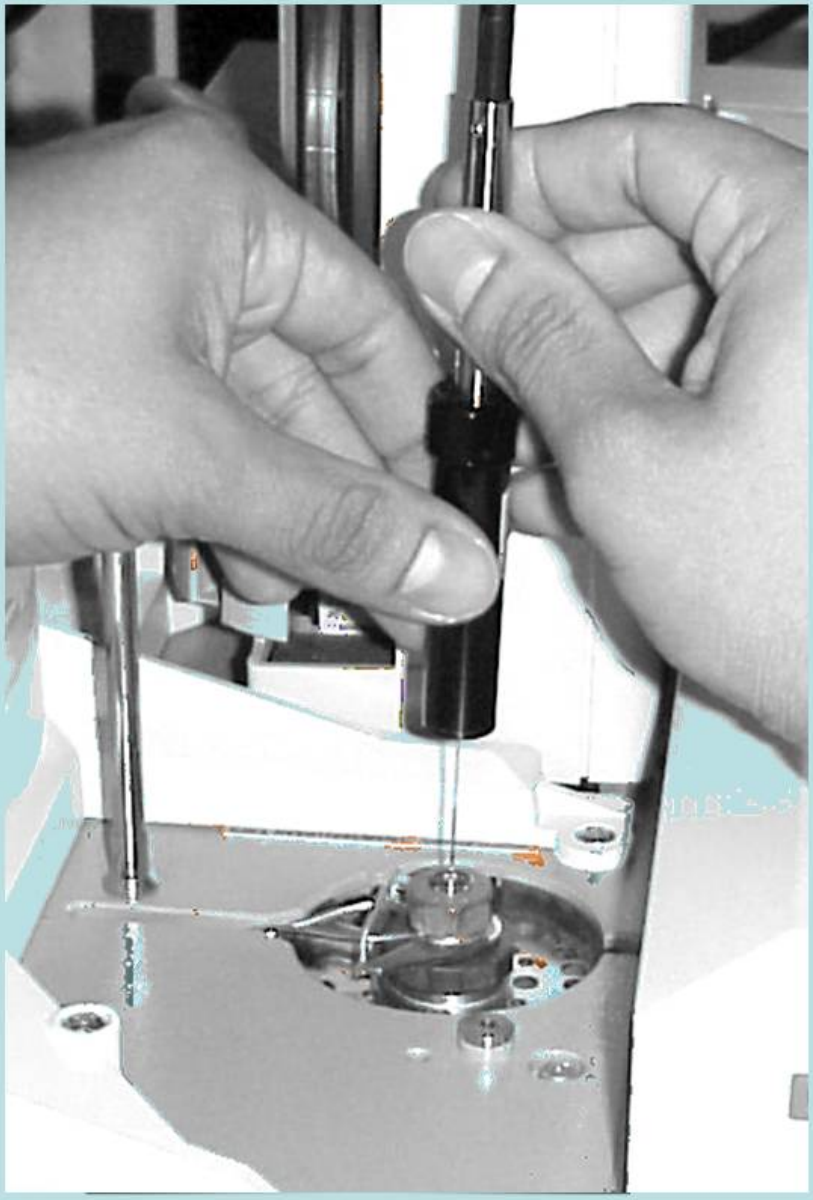
PFPH for carbonyls

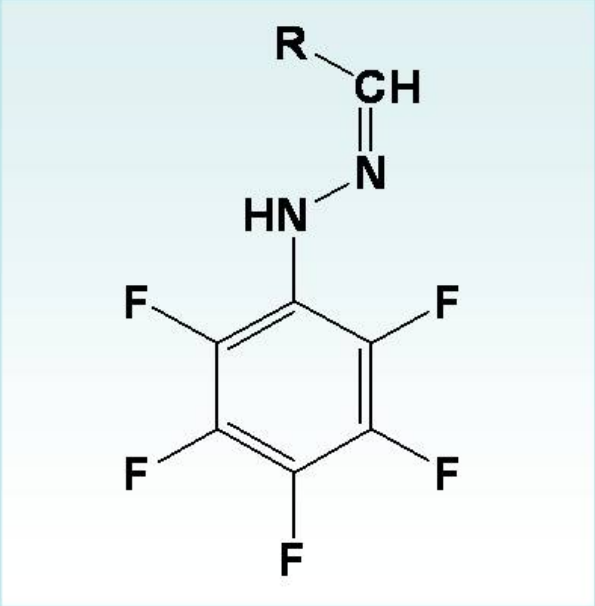


PFBBr for acids

Elena E. Stashenko *et al.*:
J. Chromatogr. Sci. **2006**, 44 (6) 347.
TrAC, **2004**, 23(4) 553.
J.. Chromatogr. A, **2004**, 1025, 99.
Anal. Bioanal. Chem., **2002**, 373, 70.
J. Chromatogr. A, **2000**, 886, 175.

On-fiber derivatisation of carbonyl compounds





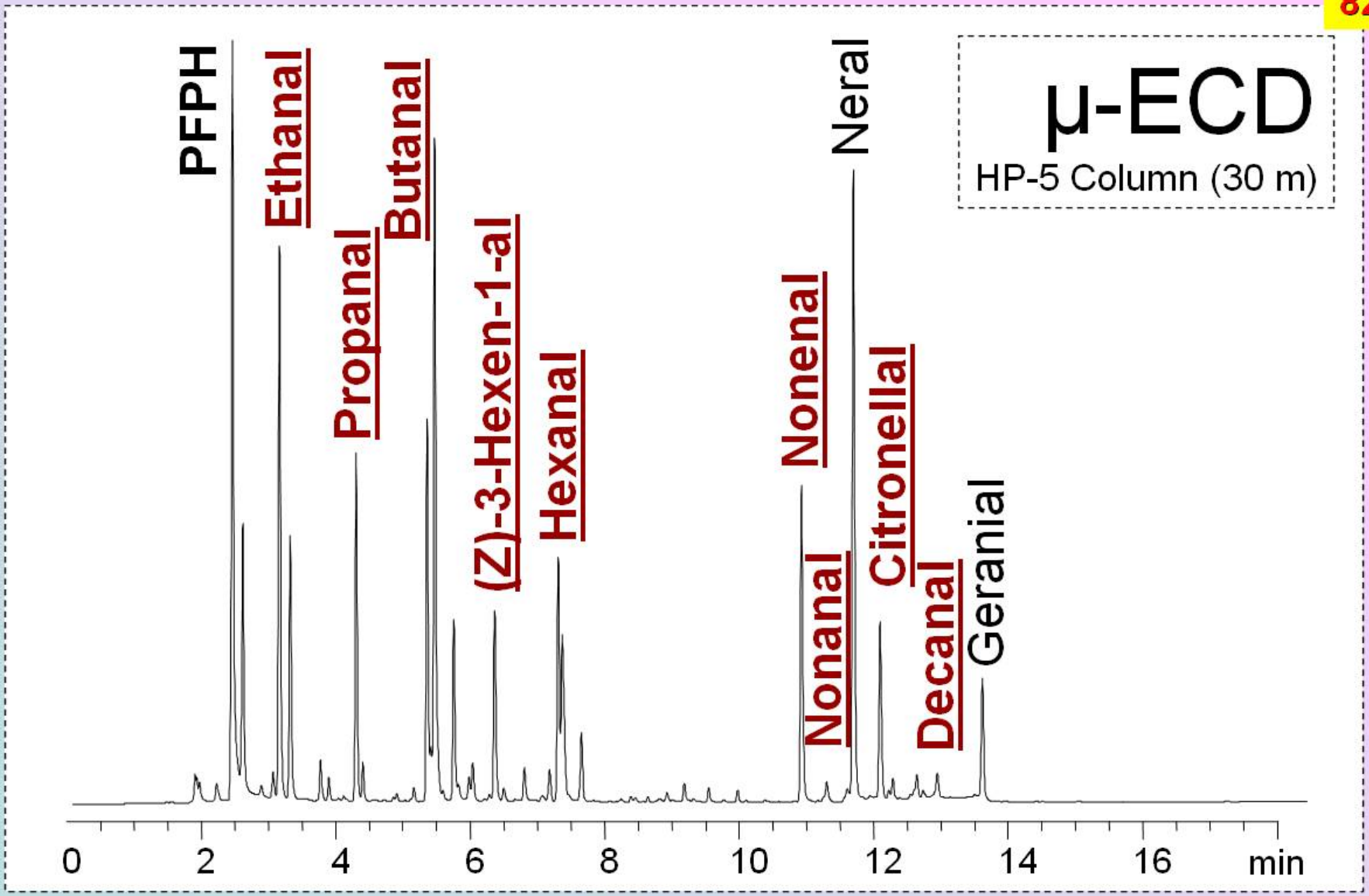
Calibration: 2 μ*M* aqueous solution of each carbonyl compound, at room temperature.

- PFPH
- Carbonyl compounds
- PFPH – derivatives

HS-SPME *in vivo* on-fibre derivatisation of carbonyls from *Swinglea glutinosa* fruits

- PFPH
- Carbonyl compounds
- PFPH – derivatives

SPME fibre saturated with PFPH



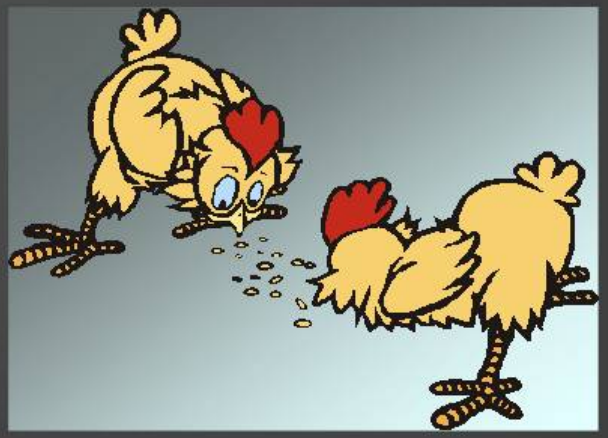
HS-SPME on-fibre derivatisation of carbonyls from *Swinglea glutinosa* fruits, monitored *in vivo*. PDMS/DVB. Exposition time 30 min.

Mode	Injection Volume	Column	Detection system	Number of components (>0.005%)
Split, 1:30	1 µL	DB-1MS, 60 m	GC-MSD, Q *full scan*	25
Splitless	1 µ L	DB-1MS, 60 m	GC-MSD, Q *full scan*	*ca.* 60
On-column	0.2 µL	DB-1MS, 60 m	GC-MSD, Q *full scan*	45
Split, 1:30	1 µL	BPX-5, 30 m	GC--TOF *full scan*	46
Split, 1:30	1 µL	1D: BPX-5, 30 m 2D: BP-20, 0.26 m	GC x GC-TOF* *full scan*	*ca.* 65
HS-SPME, *Split* 1:30	-	DB-1MS, 60 m	GC-MSD, Q *full scan*	56

"New" compounds: *e.g.* cymen-8-ol (+ terpinen-4-ol), terpinyl acetate, α-terpineol, sesquisabinene, germacrene D, bicyclogermacrene, farnesol.

Another example

Massive chicken death in poultry farms in Bucaramanga- Colombia: A detective story with chromatographic solution

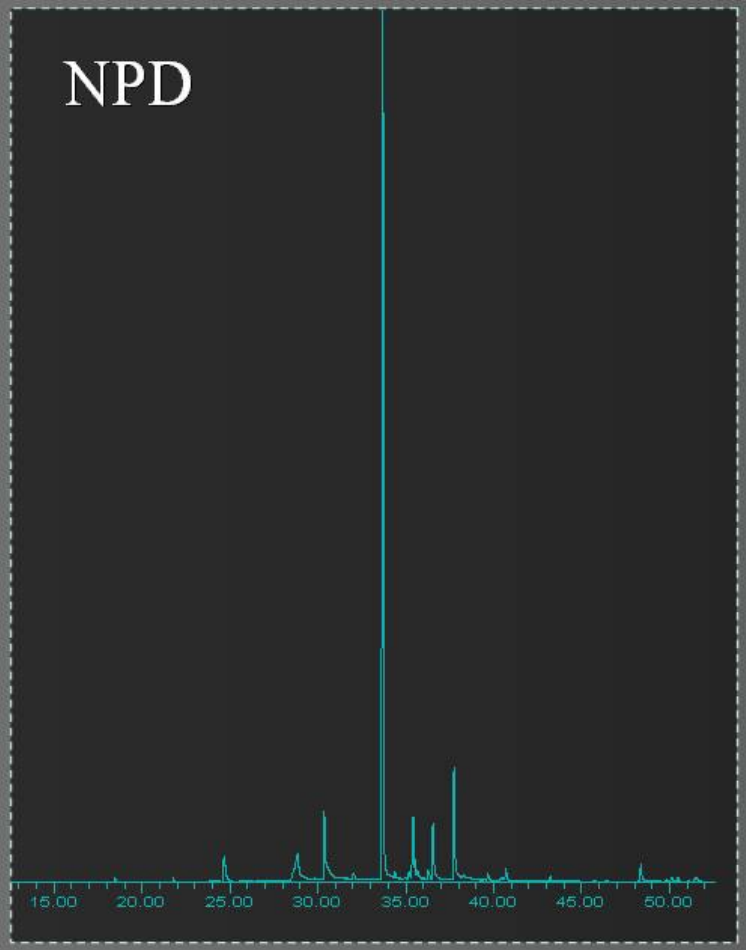


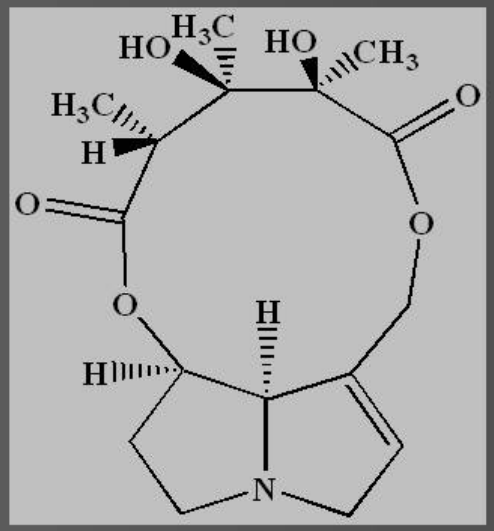
☹ Three years ago a massive chicken death occurred in several poultry farms, and was characterised by severe liver and lung damages.

😐 The cause of death was associated with the consumed food, based on sorghum grains and some additives.

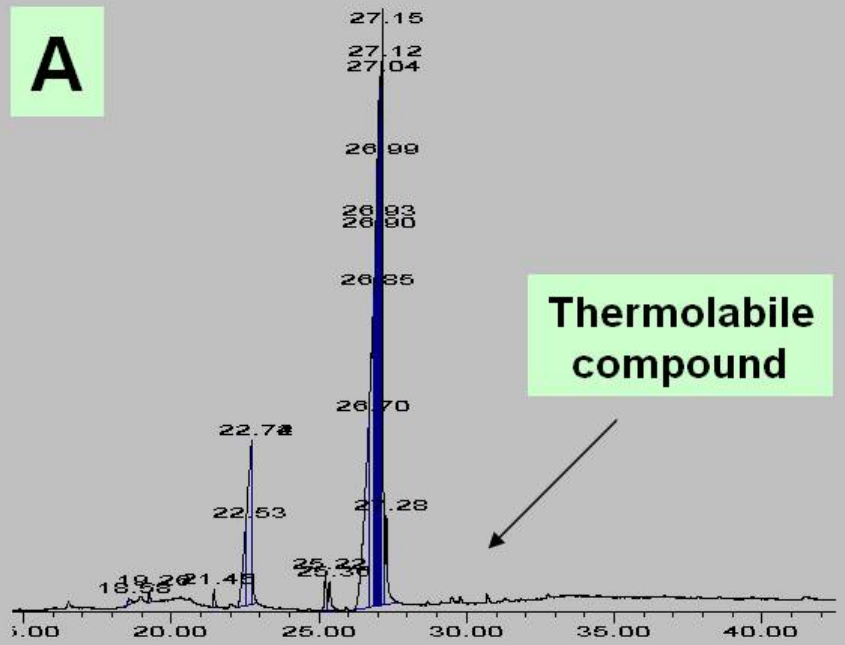
☺ The analysis of *Soxhlet-* and *SFE-* food extracts **did not reveal** the presence of any pesticide traces, aflatoxins, or bacterial contamination.

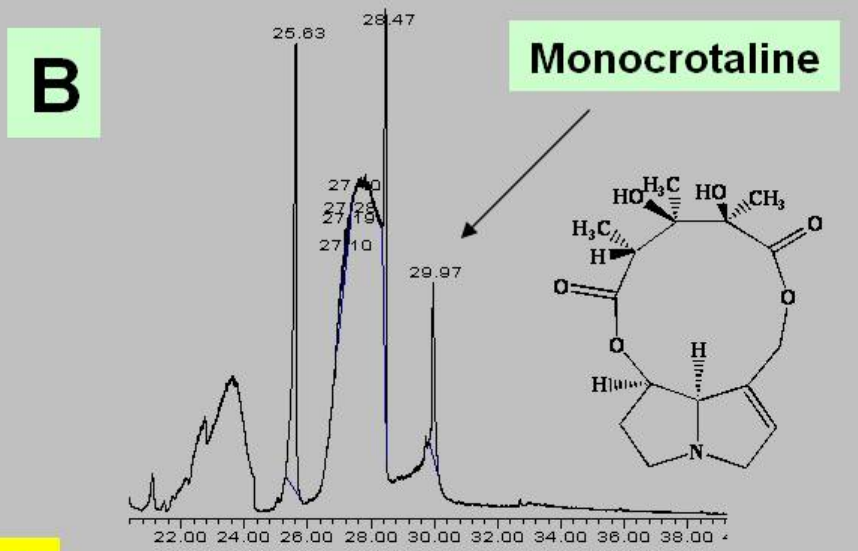
GC/NPD analysis of the Soxhlet- and SFE- extracts of chicken food showed the presence of some nitrogen-containing compound, which was isolated and analysed by IR-, ^{13}C-NMR-, XRD, and MS (EI, 70 eV)



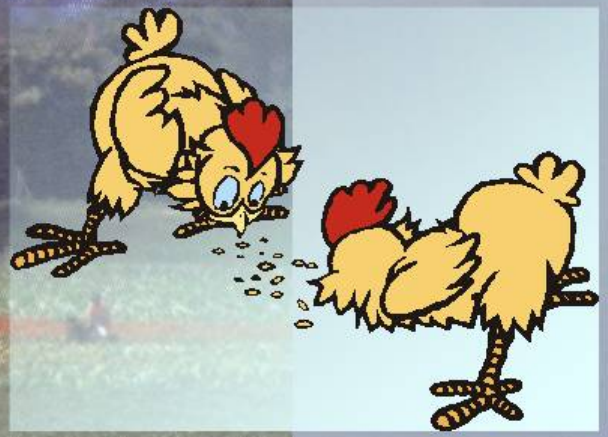


MONOCROTALINE:
substance with hepatotoxic and pneumotoxic properties.

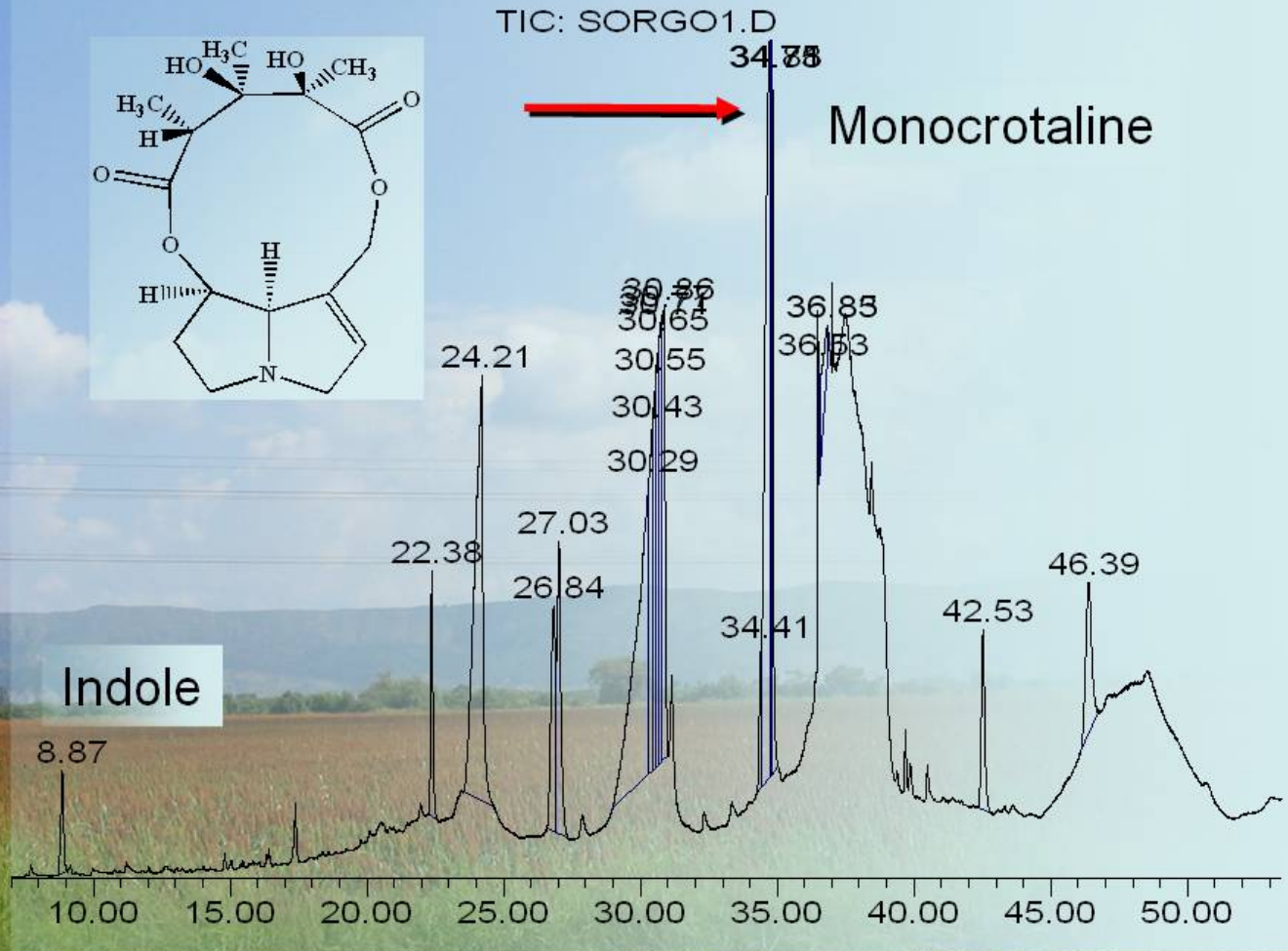

A
Thermolabile compound
27.15
27.12
27.04
26.99
26.93
26.90
26.85
26.70
22.74
22.53
27.28
20.00
25.00
30.00
35.00
40.00


B
Monocrotaline
25.63
28.47
27.10
29.97
22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00

Splitless
Pulsed splitless
A
B



Sorghum grains are the principal component for the chicken food preparation

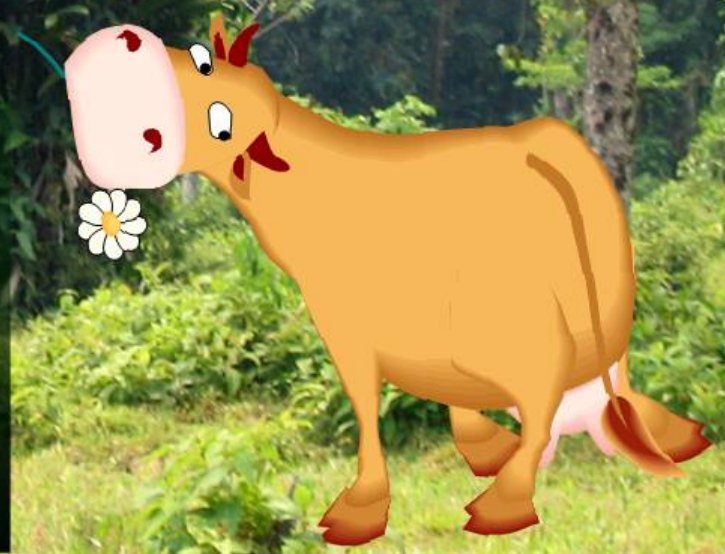


Those sorghum grains, which were used for the preparation of chicken food, contained Monocrotaline (!)

🕮 Pyrrolizidine alkaloids

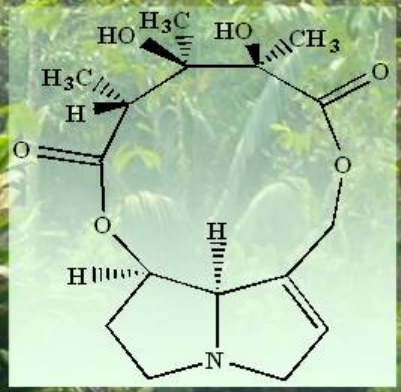
☹ Pyrrolizidine alkaloids are toxic secondary metabolites of a wide variety of plants, mainly *Compositae, Boraginacea* and *Leguminoseae* families, among some others.

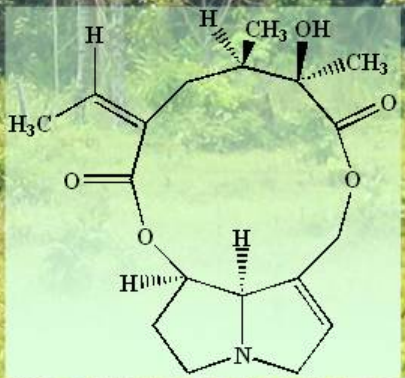
☹ They are hepatotoxins and cause death and liver damage in many farm animals.



Pyrrolizidine alkaloids

- Natural herbal teas
- Traditional folk remedies
- Grain-based foods

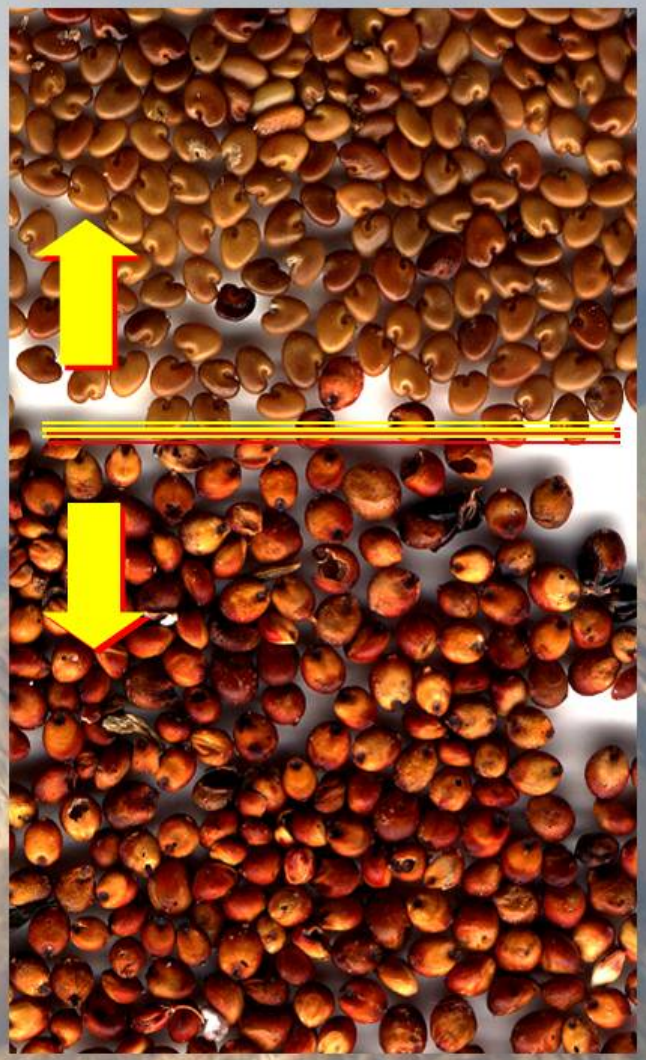


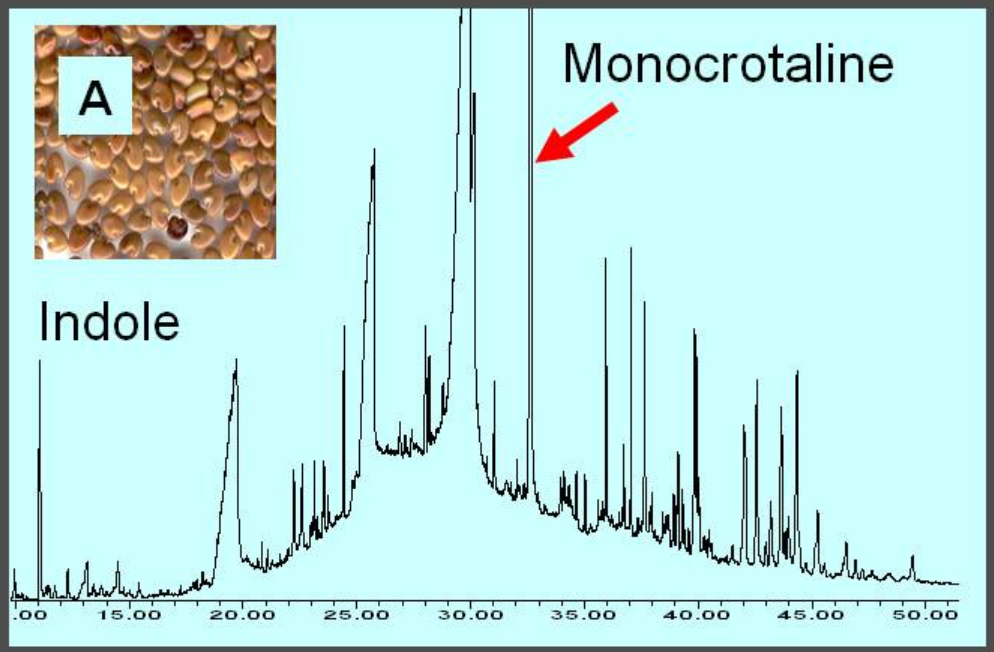


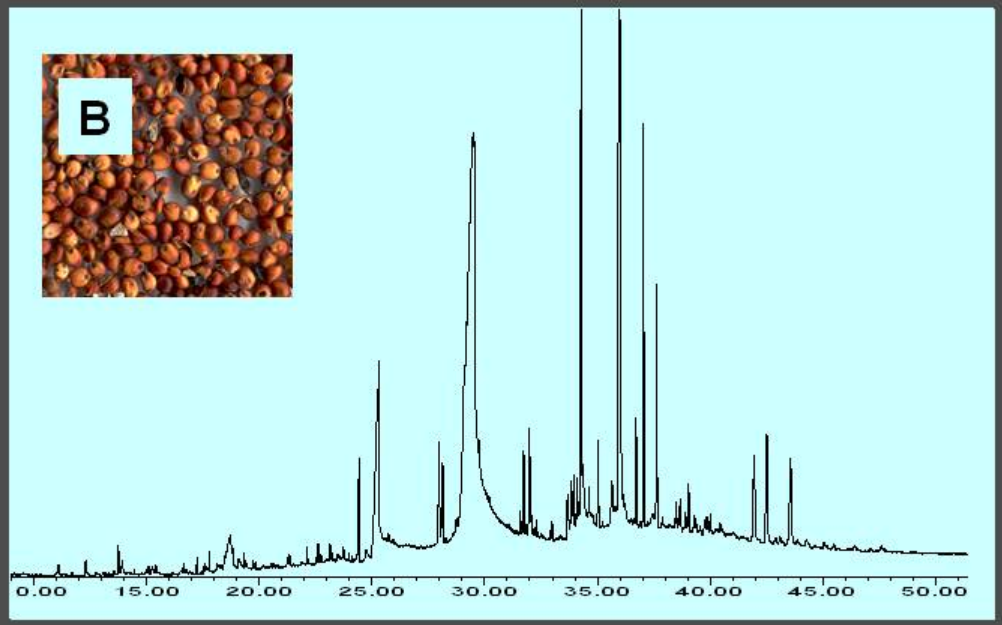
☹ Pyrrolizidine alkaloids have been identified as causing also human deaths in less developed countries, as a result of contamination of cereal crops and harvested seed, and they have been suspected of causing illness, following ingestion of some culinary vegetables or herbal remedies.

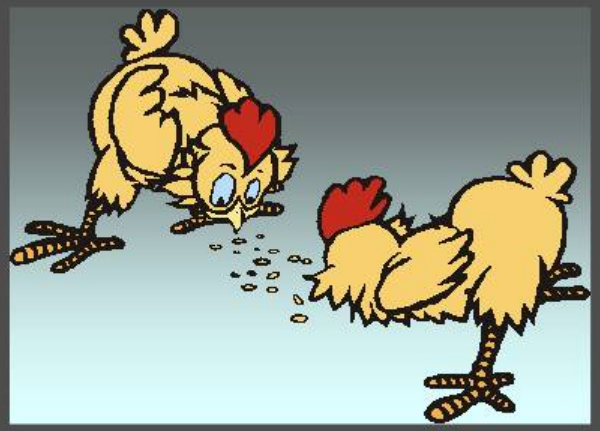
Where did the monocrotaline alkaloid in chicken food come from?

Detailed examination of the sorghum grains revealed the presence of a small percentage of seeds (*ca* 4%), with the same size, but slightly different colour and shape. The later were carefully separated and extracted both by Soxhlet and SFE. The extracts were analysed by GC-MS, and monocrotaline was identified as a major component.





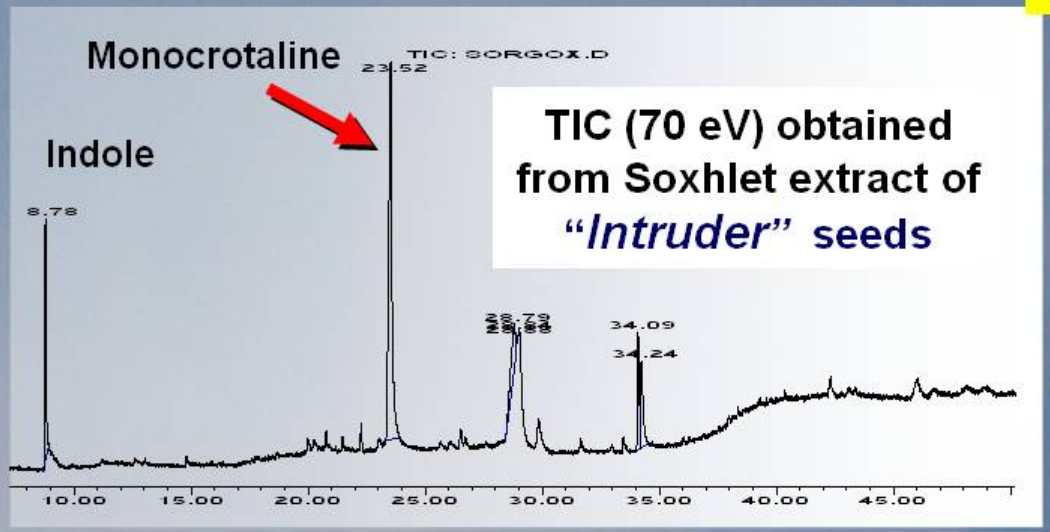




Soxhlet extracts obtained from:

A. Sorghum grains, **with** "intruder" seeds;

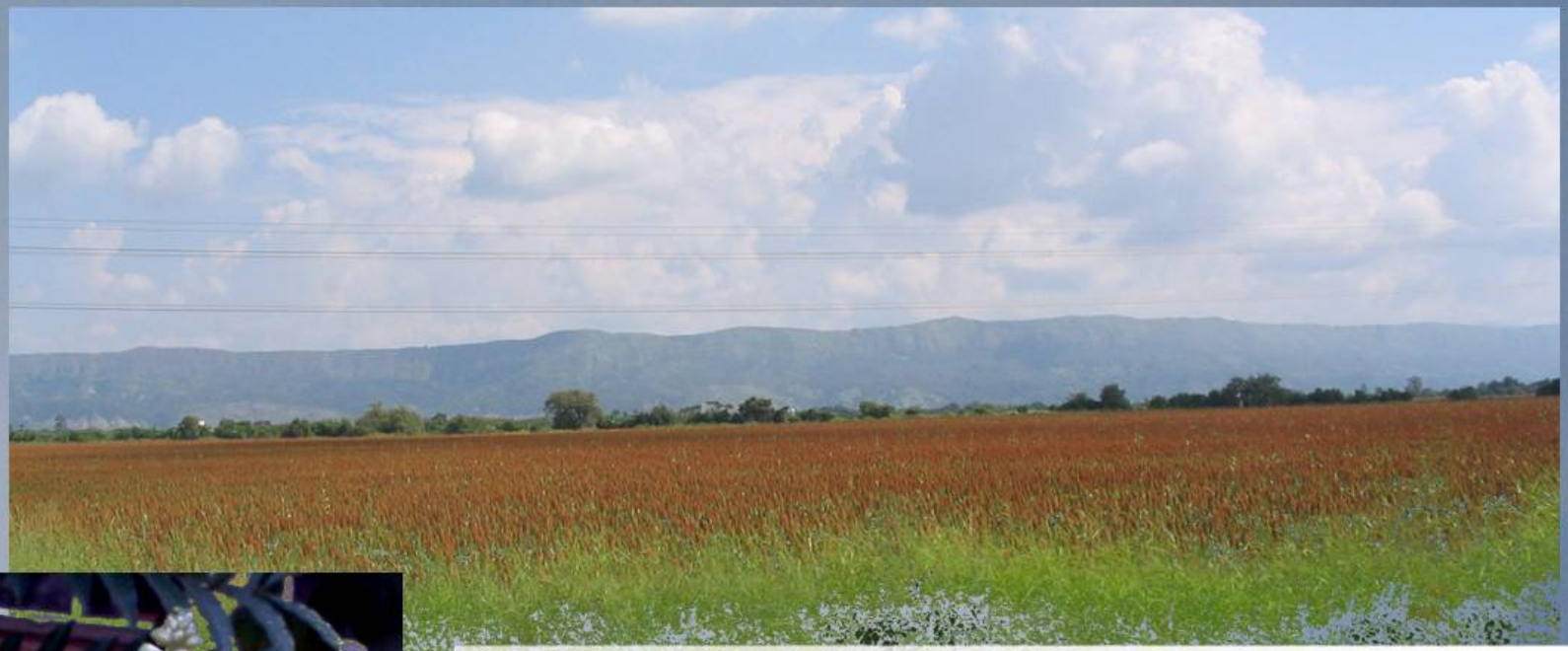
B. Sorghum grains **free** from *intruder* seeds



TIC (70 eV) obtained from Soxhlet extract of *"Intruder"* seeds

It is not so easy to determine the plant specie only on the basis of its seeds.

Thus, the seeds, separated from sorghum grains, <u>were planted and gave rise to the plant identified as</u> *Crotalaria retusa*.



Crotolaria retusa (Leguminoseae Fam.), is an effective nitrogen-fixing plant specie and it is frequently planted before sorghum in order to improve soil characteristics. Before sorghum planting, *Crotalaria retusa* plants should be removed. If this does not occur, the harvested sorghum grains are contaminated with the *Crotalaria* seeds, which contain monocrotaline, an extremely toxic hepatotoxic alkaloid.

The problem was solved:

the cause of chicken death was the intoxication with pyrrolizidine alkaloids from *Crotalaria retusa* seeds, which were mixed with sorghum grains, used for chicken food preparation.

The detailed report was sent to Colombian Ministry of Agriculture, the case was closed,

but … not the story …

Seeds, separated from sorghum grains

Crotalaria retusa
Leguminoseae Fam.

"New" seeds from plants cultivated in the University Experimental Garden

Soxhlet and SFE extractions

1 - 5

Surprise!

6

7

In the obtained both Soxhlet, and SFE extracts, no traces of pyrrolizidine alkaloids were detected!

🕮 Pyrrolizidine alkaloids play an important role in insect-plant relationships.

It was necessary to observe the *Crotalaria* sp. plants in their natural *habitat*

Caterpillars:

- ☞ *Artiidae*
- ☞ *Notodontoidae*

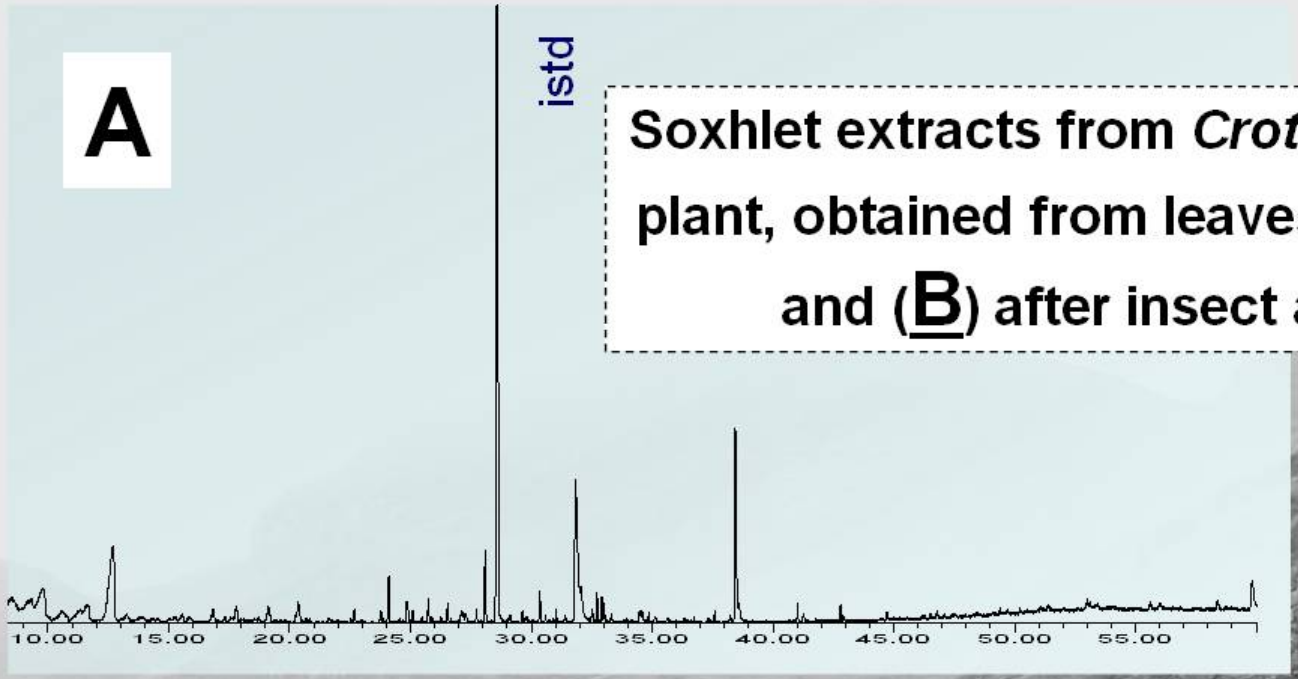
Undamaged plant:

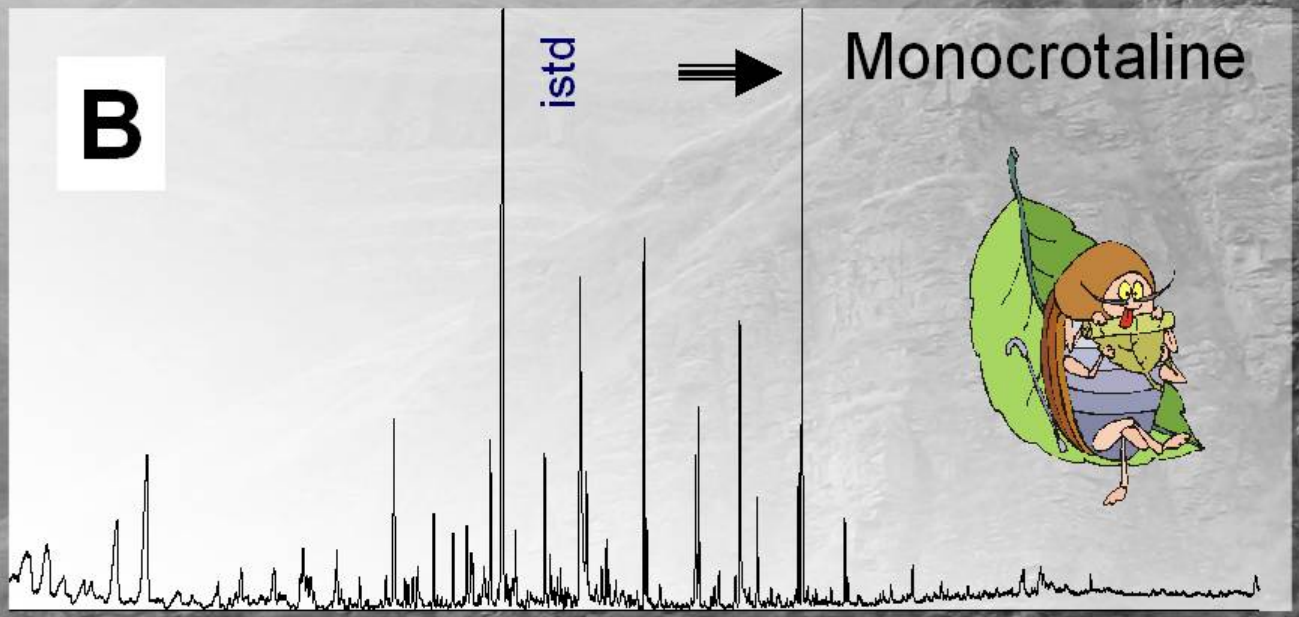
No alkaloid was detected.

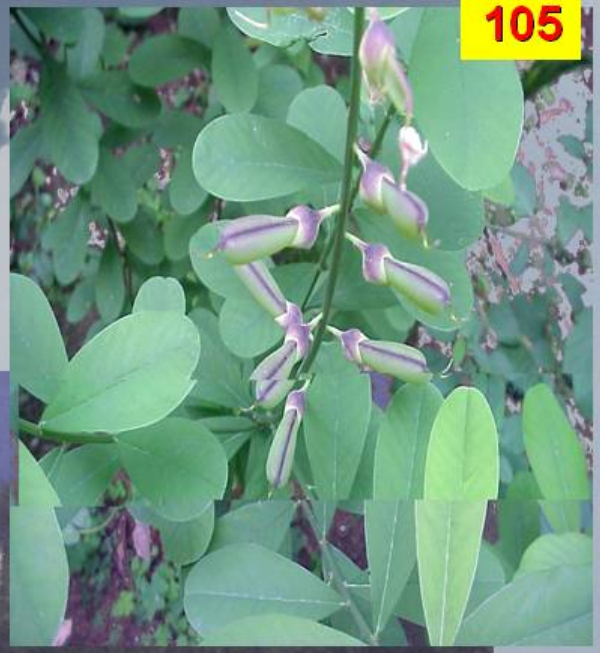
Plants attacked by *Caterpillars* :

Pyrrolizidine alkaloids were detected in large amounts.

Soxhlet extracts from *Crotalaria retusa* plant, obtained from leaves (A) before and (B) after insect attack.







END

☺ Life is joy

✌ Life is struggle

Difficulties speed up the evolution and accelerate progress

😐 Life is work, that brings happiness, wealth, dignity, and independence ☺

☺ Happiness means to share, to overcome obstacles and not to give up

$ Money can separate people ☹

☺ Chromatography – will NOT!

Many thanks and acknowledgements!

- ISC06 Organisers
- EuSSS
- Colciencias, Colombia
- Industrial University of Santander
- Prof. Dr. J. René Martínez

Jesús Olivero Vergel - Amanda L. Mora Martínez - Marianny Yajaira Combariza - Gladys Checira Hernández - Gloria Inés García Jerez - Yolanda Osorio Villamizar - Martín Augusto Escobar Hernández - Ricardo Acosta Amado - María del Pilar Tirado - William Torres Delgado - Sandra Liliana Estévez - Hernán Vicente Fuentes Patrón - Martha Cervantes Díaz - Fernando José Hernández Blanco - Luis Gonzalo Sequeda Castañeda - Sandra Natalia Correa Torres - Andrea Rangel Camargo - Mónica Eliana Niño Porras - Alix Marcela Murcia - Hans Thielin Castro Salazar - Sonia Carolina Rodríguez Amaya - Fausto Prada Chaparro - José Domingo Medina Sarmiento - Elkin Dario Rueda Parra - José Luis Molina Gevarra - Raúl Rodríguez - Martha Barrera - Libia Fonseca García - Tatiana Tsarkova - José Carlos Gutiérrez - Jorge Saúl Ramírez A. - Luz Ángela Carreño Díaz - María Constanza Ferreira Luna - Fulvia Nydia Sánchez Leal - Cesar Roberto Martínez - Miguel Angel Puertas - Hiltony Villa Dangond - Juan Carlos Santos - Cristian Blanco Tirado - Nubia Quiroz Prada- María del Pilar Garcés Arias - Crispín Astolfo Celis Zambrano - Dora Cecilia Rodríguez Ordóñez - Elizabeth Gil Archila - Wilman Delgado Avila - Elena Losik - Judith Rocio Santa Jaimes - Martín Serrano Ardila - Juan Pablo Arrubla Vélez - Nelson Contreras Coronel - Geovanna Tafurt García - Alba Teresa Álvarez Martínez - Doris Gutiérrez Hernández - Maritza Villalobos B. - Amner Muñoz Acevedo - Deyanira Caballero Mendez

Thank you very much!