



Master Biennale di II livello in  
Discipline Regolatorie "G. Benzi"



FEDERCHIMICA  
ASCHIMFARMA

Associazione nazionale produttori principi attivi  
e intermedi per l'industria farmaceutica



Associazione Farmaceutici Industria



Master in Tecnologie Farmaceutiche e Attività Regolatorie  
Università di Pavia

## WORKSHOP

23<sup>rd</sup> November, 2018

### **ASMF: GMP perspective and regulatory compliance from starting materials to API**

*ASMF: aspetti GMP e criticità regolatorie dallo starting material alla sostanza attiva*

Aula Magna – Collegio A. Volta  
Via Adolfo Ferrata, 19 – Pavia (PV)  
University of Pavia (Italy)

# Modern Trends in Quality and Regulatory Aspects of Herbal Extracts

Frnesto Marco Martinelli – Roberto Pace

Indena S.p.A. - Milano –Italy

Pavia, 23 Novembre 2018

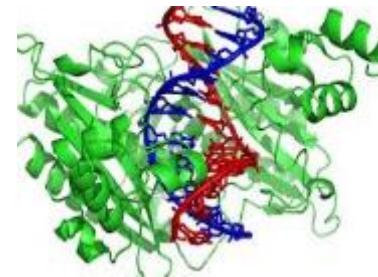




# WHAT PLANTS CONTAIN

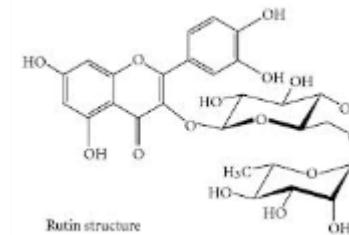
PLANT ARE INCREDIBLE VARIABLE SOURCES  
OF SUBSTANCES THAT ARE GENERALLY DIFFICULT TO SYNTHETIZE  
DUE TO THEIR CHEMICAL COMPLEXITY  
CAN BE CLASSIFIED AS

- PRIMARY METABOLITES



AND

- SECONDARY METABOLITES



# PRIMARY VS SECONDARY PLANT METABOLISM

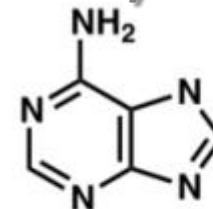
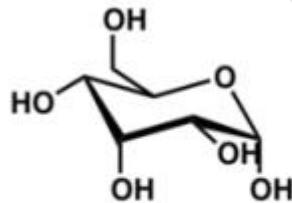
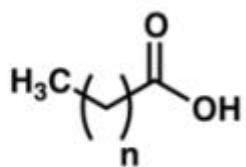
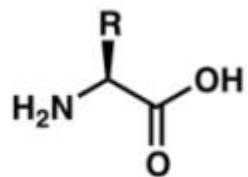
PRIMARY METABOLISM IN A PLANT COMPRISES ALL METABOLIC PATHWAYS THAT ARE ESSENTIAL TO THE PLANT'S SURVIVAL

PRIMARY METABOLITES ARE COMPOUNDS THAT ARE DIRECTLY INVOLVED IN THE GROWTH AND DEVELOPMENT OF A PLANT

SECONDARY METABOLITES ARE COMPOUNDS PRODUCED IN OTHER METABOLIC PATHWAYS THAT, ALTHOUGH IMPORTANT, ARE NOT ESSENTIAL TO THE FUNCTIONING OF THE PLANT

ARE USEFUL IN THE LONG TERM, OFTEN FOR DEFENCE PURPOSES  
SECONDARY PLANT METABOLITES ARE ALSO USED IN SIGNALLING AND REGULATION OF PRIMARY METABOLIC PATHWAYS

# PRIMARY METABOLITES (EXAMPLES)



amino acids

fatty acids

carbohydrates

nucleobases

glycerol

mono-saccharides

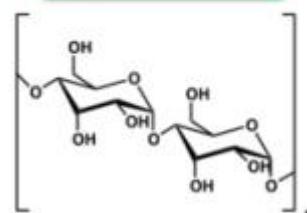
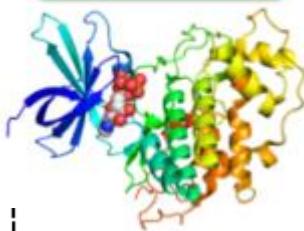
nucleotides

proteins

phospholipids

poly-saccharides

DNA / RNA

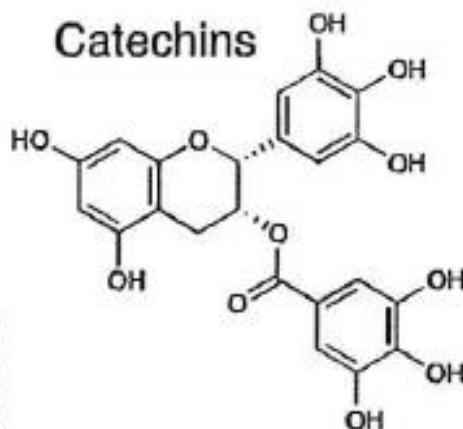




# SECONDARY METABOLITES (EXAMPLES)

Phenolics, phenylpropanoids  
and polyketides

Catechins



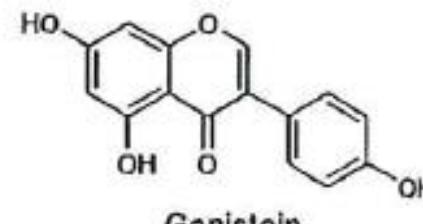
Epigallocatechin gallate

Flavones



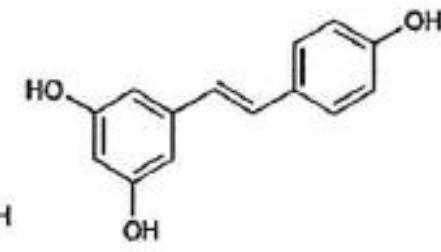
Quercetin

Isoflavones



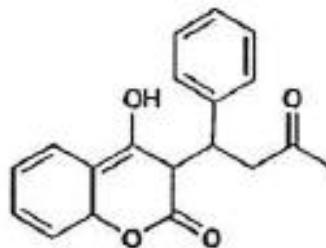
Genistein

Stilbenes



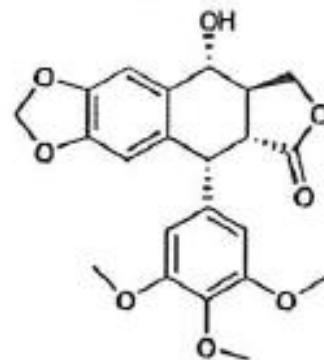
Resveratrol

Coumarins



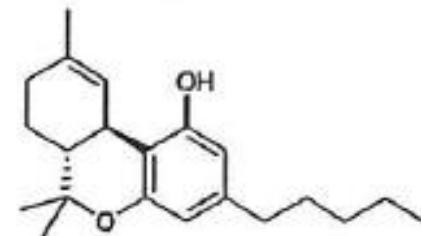
Warfarin

Lignans



Podophyllotoxin

Polyketides



Tetrahydrocannabinol

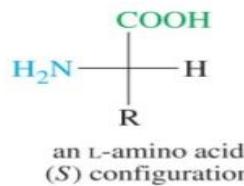
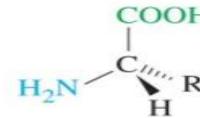
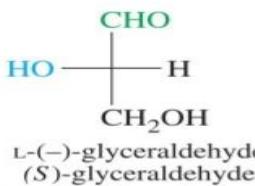
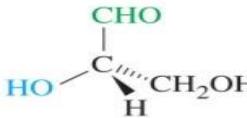
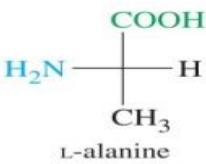
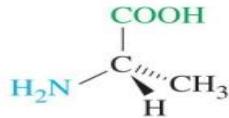


# THE PLANTS CONTAIN SUBSTANCES WITH THE FOLLOWING ATTRIBUTES

- GENERALLY COMPLEX CHEMICAL STRUCTURE
- MANY SUBSTANCES BELONGING TO THE SAME CLASS (FLAVONOIDS, FLAVONS, SESQUITERPENES ETC.)
- ARE PRESENT IN A CHARACTERISTIC PATTERN OF CONSTITUENTS IN THE SAME PLANT/PART OF THE PLANT/MATURATION STAGE
- NATURAL UNIQUE DEFINED STEREOCHEMISTRY

Example:

## Stereochemistry of $\alpha$ -Amino Acids

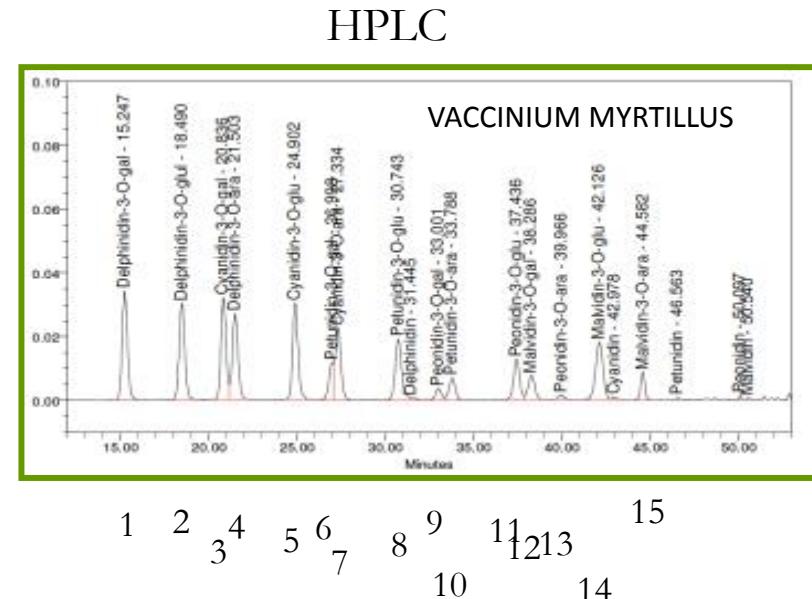
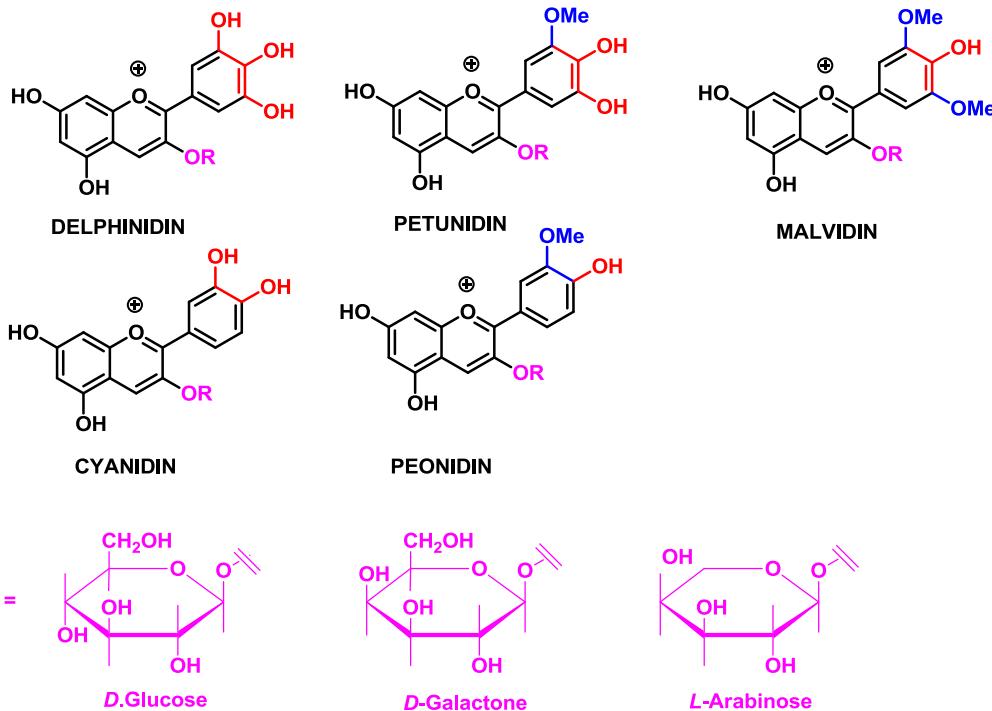


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# THE PLANTS CONTAIN SUBSTANCES WITH THE SAME PATTERN (EXAMPLE)

## Anthocyanins of *V. myrtillus* dry extract

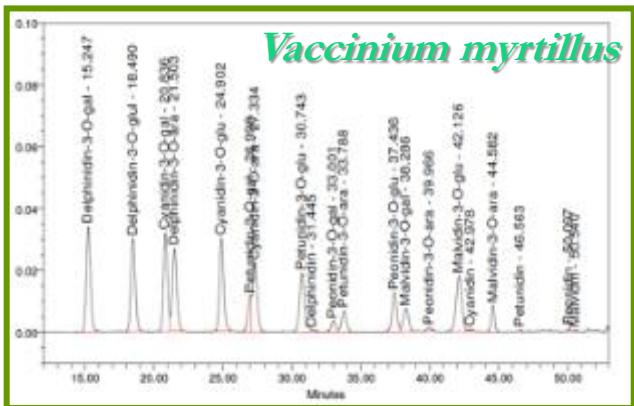


15 Anthocyanins with characteristic fingerprint

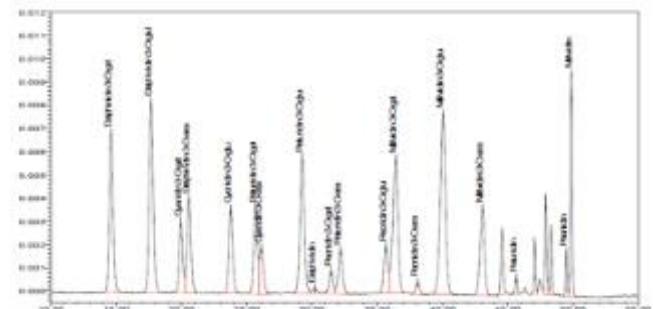
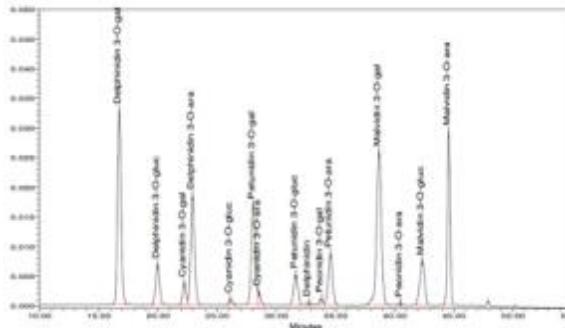


# THE PLANTS CONTAIN SUBSTANCES WITH THE SAME PATTERN

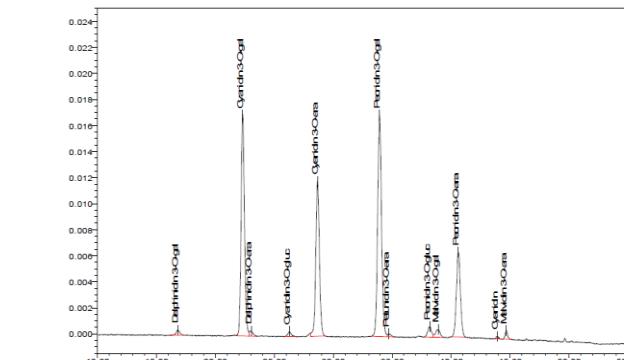
e.g. Vaccinium myrtillus anthocyanins have a very characteristic HPLC pattern that allows to distinguish the other berries.



Vaccinium corymbosum, Highbush blueberry



Vaccinium angustifolium, Lowbush blueberry



Vaccinium macrocarpon, American cranberry

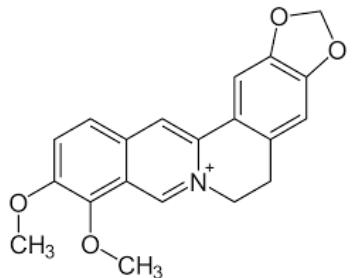


# SYNERGY 1+1=3

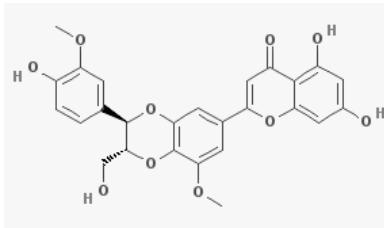
## SYNERGIC EFFECTS

MANY DATA SHOWS THAT BIOLOGICAL ACTIVITY OF THESE PREPARATIONS MIGHT RESULT FROM **SYNERGY** OF ACTIVE COMPOUNDS RATHER THAN FROM A SINGLE CHEMICAL ENTITY.

EXAMPLE



THE ANTIMICROBIAL ACTIVITY OF ALKALOID **BERBERINE** IS 100 TIMES ENHANCED BY **5'-METHOXYHYDNOCARPIN** (5'MHC).

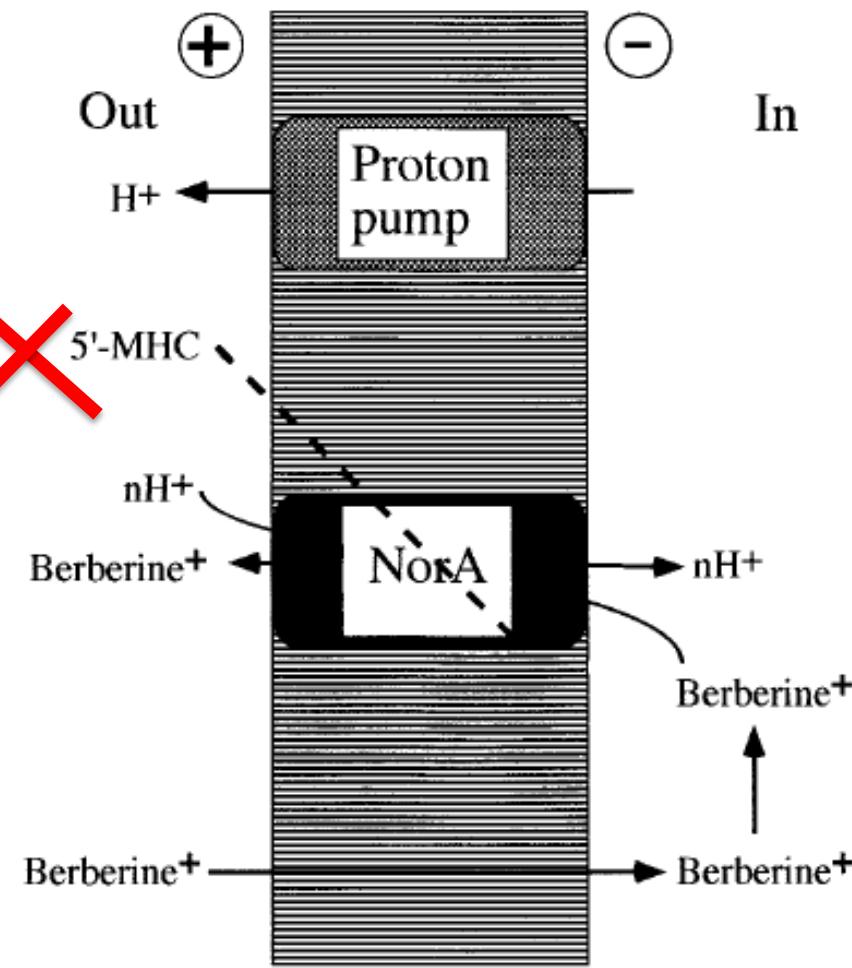
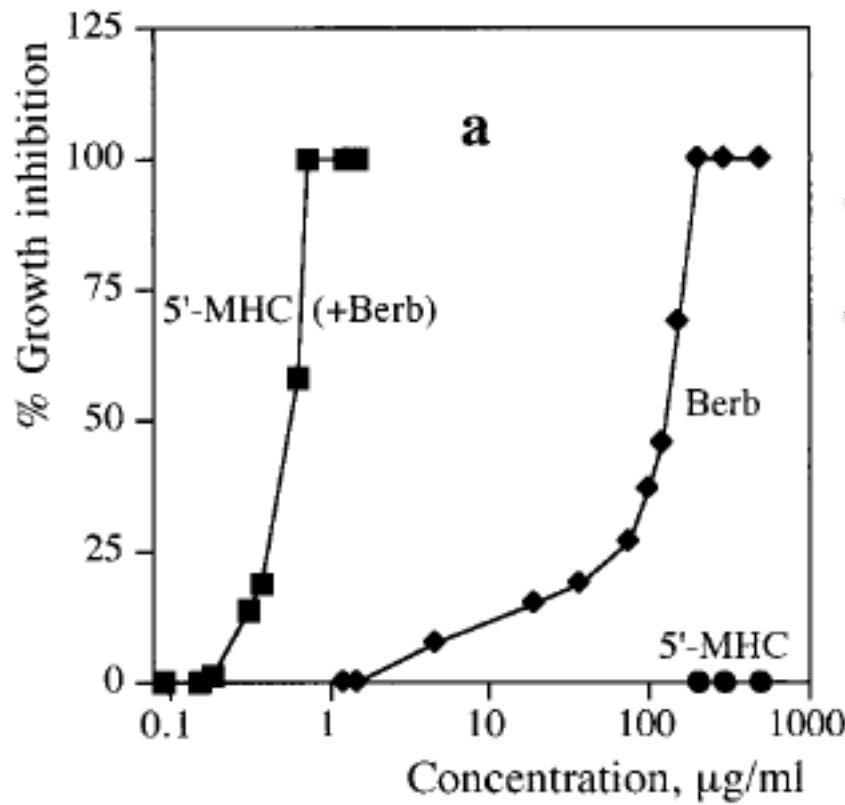


# Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin,

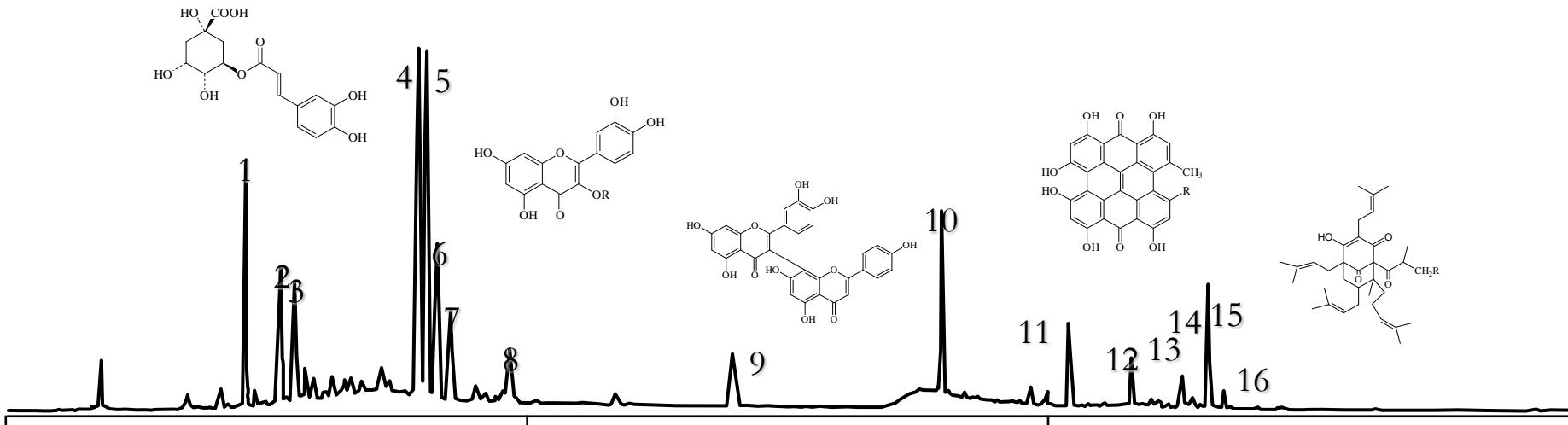
Frank R. Stermitz\*, Peter Lorenz\*, Jeanne N. Tawara\*, Lauren A. Zenewicz†, and Kim Lewis††

\*Department of Chemistry, Colorado State University, Fort Collins, CO 80523; and †Biotechnology Center, Tufts University, Medford, MA 02155

PNAS | February 15, 2000 | vol. 97 | no. 4 | 1433–1437



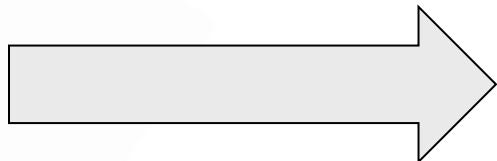
# HERBAL EXTRACTS



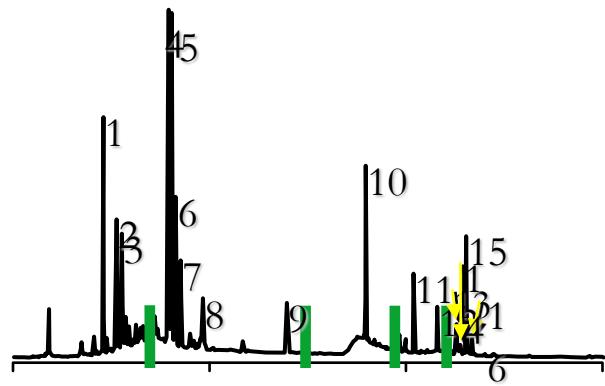
Generally it is not possible to identify a substance responsible for the biological activity: all substances generally contribute in a synergistic way to pharmacological effect and / or mitigating toxic effects.

**Isolated individual constituents generally behave in different way.**

# HERBAL EXTRACTS



They are active **ingredients**  
in their entirety



ESTRATTI DI ORIGINE VEGETALE

HERBAL DRUG/STARTING HERBAL DRUG





07/2017:1433

## HERBAL DRUGS

### Plantae medicinales

#### DEFINITION

Herbal drugs are mainly whole, fragmented or broken plants or parts of plants in an unprocessed state, usually in dried form but sometimes fresh. In this general monograph, the word 'plant' is used in the broader sense to also include algae, fungi and lichens. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs. Herbal drugs are precisely defined by the botanical scientific name according to the binominal system (genus, species, variety and author).

# IMPURITIES FOR HERBAL DRUG

The Quality of the Starting Herbal Drug should be assured by an adequate Supplier Qualification to guarantee the GACP compliance (“Good Agriculture and Collection Practice”).

London, 20 February 2006  
Doc. Ref. EMEA/HMPC/246816/2005

COMMITTEE ON HERBAL MEDICINAL PRODUCTS  
(HMPC)

GUIDELINE ON GOOD AGRICULTURAL AND COLLECTION PRACTICE (GACP) FOR  
STARTING MATERIALS OF HERBAL ORIGIN

GACP

1. ADEQUATE QA SYSTEM FOR HERBAL EXTRACTS
2. IN WILD HABITAT POSSIBLE CONFUSION DURING COLLECTION
2. QUALITY AGREEMENTS (ACCORDING TO REGIONAL REGULATIONS)
3. PERSONNELL
4. BUILDING
5. EQUIPMENT
6. DOCUMENTATION
7. SEEDS AND PROPAGATION MATERIAL
8. CULTIVATION
9. COLLECTION
10. HARVEST
11. PRIMARY PROCESSING
12. PACKAGING
13. STORAGE AND DISTRIBUTION

# ✓ Biological activity supported by Consistency of the HERBS CONSTITUENTS

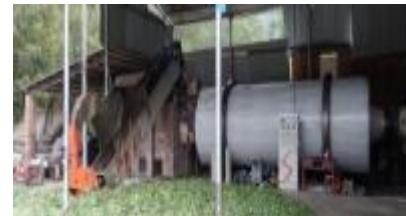
Traceability and Authenticity are Key elements to support the Consistency of the composition of the Herbs



Geographical Origin



Cultivation  
Harvesting  
(FARMERS)



Drying Process



Storage  
(including  
freezing)



Transportation

## ESTRATTI DI ORIGINE VEGETALE

### (Starting) HERBAL DRUG

a) Definition: a qualitative statement of the botanical source, plant part used and its state (e.g. whole, reduced, powdered, fresh, dry). It is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.



Genere

(Es. *Vaccinium myrtillus L.* frutti freschi (congelati))

Origine: Europa

Specie

Autore Parte della pianta

b) Characters: a qualitative statement about the organoleptic character(s) where characteristic and the macroscopic and microscopic botanical characters of the herbal substance.

Esempio:

**CHARACTERS**

*Appearance*: bright yellow-brown, powder or friable mass.

c) Identification: identification testing optimally should be able to discriminate between related species and/or potential adulterants/substitutes, which are likely to be present. Identification tests should be specific for the herbal substance and are usually a combination of three or more of the following:

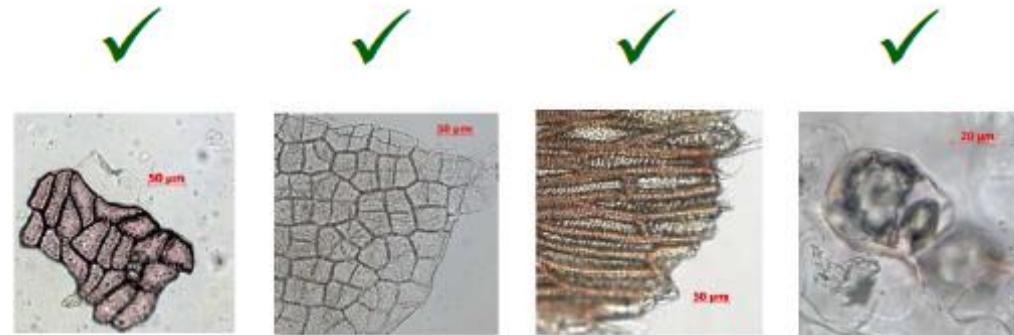
Macroscopical characters, Microscopical characters, Chromatographic procedures, Chemical reactions.

# (STARTING) HERBAL DRUG IDENTIFICATION

macroscopic id



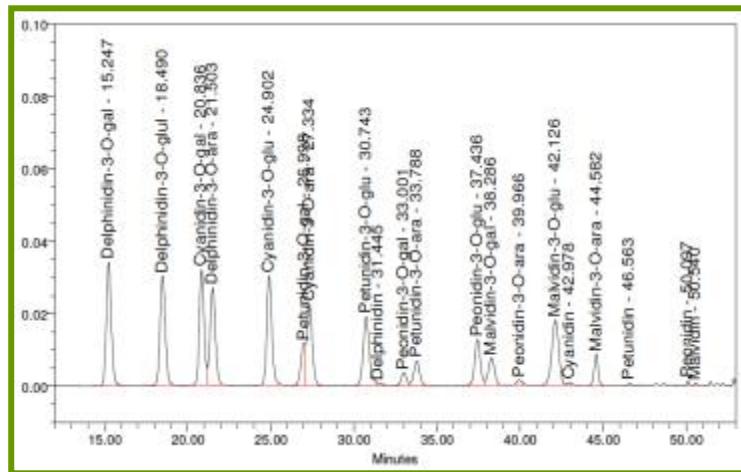
microscopic id



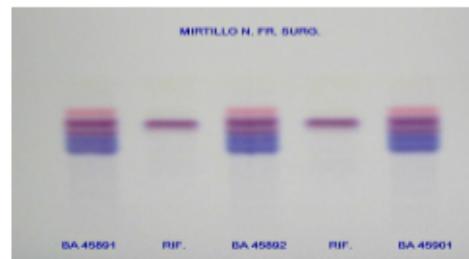
*vaccinium myrtillus l.* – bilberry

# (STARTING) HERBAL DRUG IDENTIFICATION

HPLC



TLC



*vaccinium myrtillus l.* - bilberry

d) Tests:

Foreign matter.

Total ash.

Ash insoluble in hydrochloric acid<sup>4</sup>.

Water soluble extractive<sup>4</sup>.

Extractable matter<sup>4</sup>.

<sup>4</sup> These tests might not apply to all herbal substances and must be justified by the applicant.



## ESTRATTI DI ORIGINE VEGETALE (Starting) HERBAL DRUG

Particle size: For some herbal substances intended for use in herbal teas or solid herbal medicinal products, particle size can have a significant effect on dissolution rates, bioavailability, and/or stability. In such instances, testing for particle size distribution should be carried out using an appropriate procedure, and acceptance criteria should be provided. Particle size can also affect the disintegration time of solid dosage forms.

### Commento:

La dimensione del materiale vegetale è generalmente più importante quando ci si riferisce a degli “herbal tea” ma generalmente non quando si estrae con solventi il materiale vegetale se l'estrazione viene condotta in condizioni di esaurimento del materiale vegetale.

Water content: This test is important when the herbal substances are known to be hygroscopic. For non-pharmacopoeial herbal substances, acceptance criteria should be justified by data on the effects of moisture absorption. A Loss on drying procedure may be adequate; however, in some cases (essential-oil containing plants); a detection procedure that is specific for water is required.

**Commento:**

La presenza di umidità residua nel materiale vegetale è importante in quanto un basso contenuto di acqua (< 10-12%) evita la formazione di micotossine e favorisce la conservazione dei markers attivi.

- 
- e) Assay: In the case of herbal substances with constituents of known therapeutic activity or with active markers, assays of their content are required with details of the analytical procedure. Where possible, a specific, stability-indicating procedure should be included to determine the content of the herbal substance. In cases where use of a non-specific assay is justified, other supporting analytical procedures may be used to achieve overall specificity if required.

In the case of herbal substances where the constituents responsible for the therapeutic activity are unknown assays of analytical markers or other justified determinations are required. The appropriateness of the choice of markers should be justified. For example, reference to the assay of a marker in the relevant monograph of the European Pharmacopoeia is an appropriate justification.



# Controlli tipici di un Herbal Drug (esempio di una Monografia della Farmacopea Europea, Ginkgo biloba foglie)

## GINKGO LEAF

### Ginkgonis folium

#### DEFINITION

Whole or fragmented, dried leaf of *Ginkgo biloba* L.

HPLC → *Content*: not less than 0.5 per cent of flavonoids, expressed as flavone glycosides ( $M_r$  757) (dried drug).

#### IDENTIFICATION

**A. Macroscopic description** The leaf is greyish or yellowish-green or yellowish-brown. The upper surface is slightly .....

**B. Microscopic examination (2.8.23)**. The powder is greyish or yellowish-green or yellowish-brown. Examine under a microscope .....

**C. Thin Layer Chromatography (2.2.27)** .....

#### TESTS

**Foreign matter (2.8.2)**: maximum 5 per cent of stems and 2 per cent of other foreign matter.

**Loss on drying (2.2.32)**: maximum 11.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (2.9.12) by drying in an oven at 105 °C for 2 h.

**Total ash (2.4.16)**: maximum 11.0 per cent.

# IMPURITIES FOR HERBAL DRUG (contaminants)

CONTAMINANTS



Foreign Matter

Pesticides

Heavy Metals



Mycotoxins

Irradiation

HERBAL DRUG



Pyrrolizidine  
Alkaloids/Tro-  
pan Alk.

Microbial



Polycyclic  
Arom.  
Hydrocar.





# Controlli tipici di un Herbal Drug (esempio di una Monografia della Farmacopea Europea, Ginkgo biloba foglie)

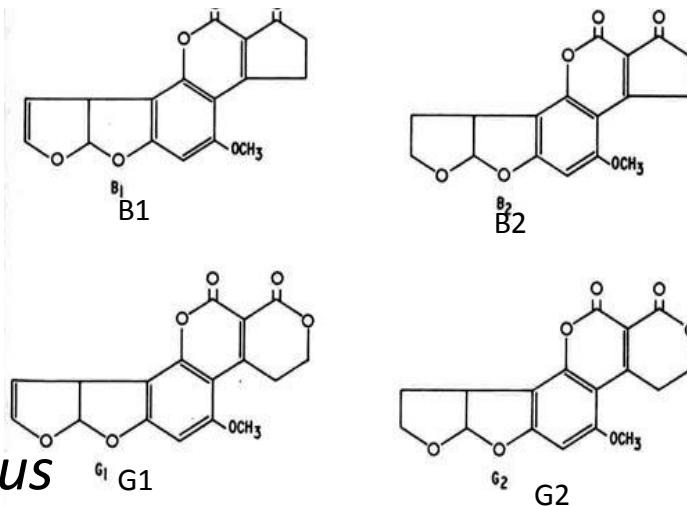
## CONTAMINANTS

- Heavy Metals (According to European Pharmacopoeia)  
**Pb** < 5 ppm  
**Cd** < 1 ppm  
**Hg** < 0.1 ppm  
**As**
- Pesticides (According to European Pharmacopoeia 2.8.13)
- Aflatoxins/Ochratoxins (According to European Pharmacopoeia 2.8.18 ; 2.8.22)  
< 2 ug/Kg (B1);  
< 4 ug/Kg (sum B1 B2 G1 G2)
- Pyrrolizidin Alkaloids/Tropane Alkaloids (recently added).

# Controlli tipici di un Herbal Drug

## CONTAMINANTS: MYCOTOXINS

- Mycotoxins (aflatoxins, ochratoxin A): The potential for mycotoxins contamination should be fully considered. For aflatoxins, procedure and acceptance criteria should follow pharmacopoeial precedents (2.8.18) and for ochratoxin A, the procedure is described (2.8.22) and acceptance criteria are given in specific monographs.



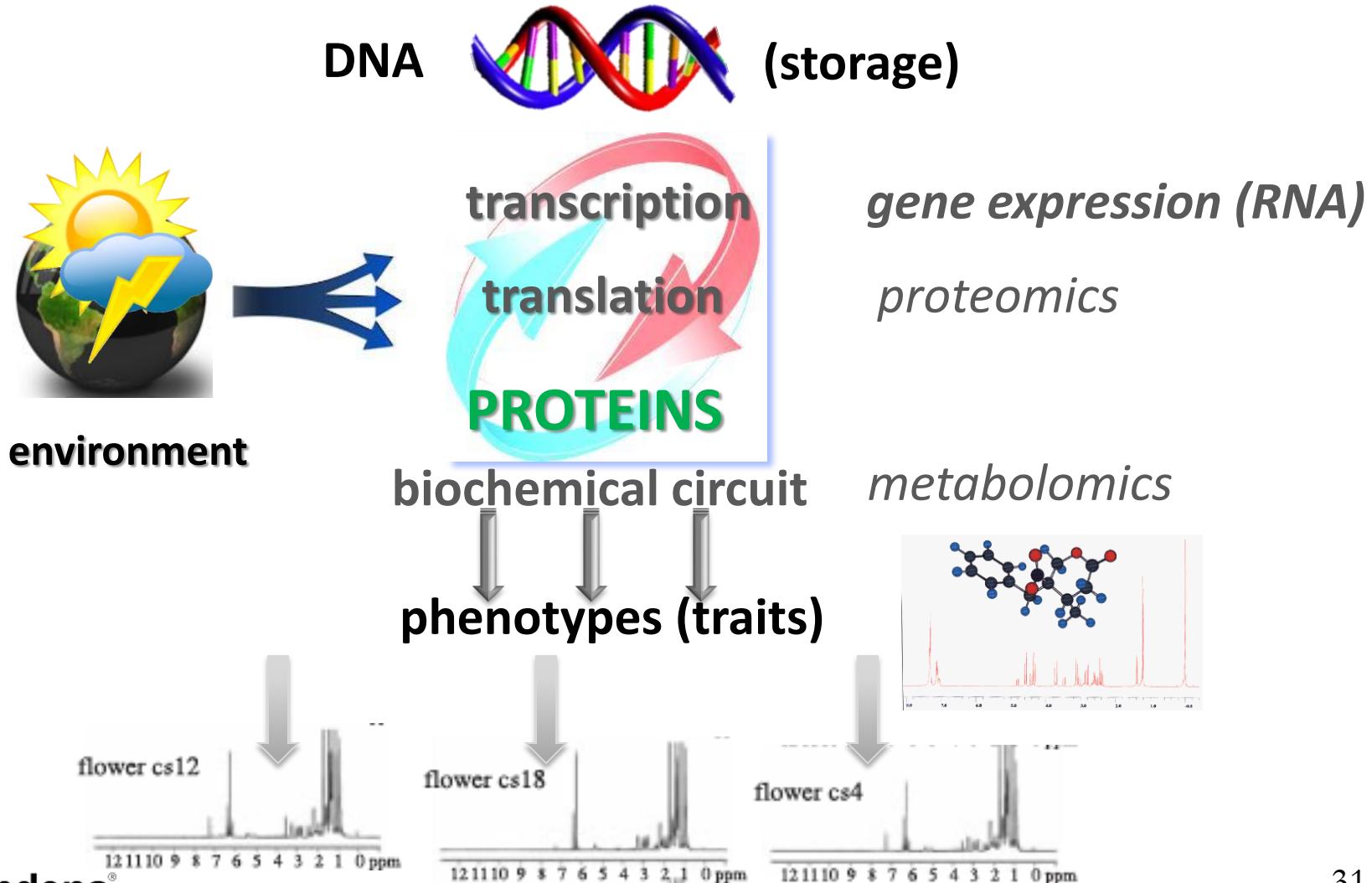
e.g. *Aspergillus flavus*

Fig. 1 Structures of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>.

Among the mycotoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> e G<sub>2</sub>) are the most widespread and dangerous for their genotoxicity .

Unless otherwise indicated in the monograph, herbal drugs contain not more than 2 µg/kg of aflatoxin B<sub>1</sub>. The competent authority may also require compliance with a limit of 4 µg/kg for the sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>.

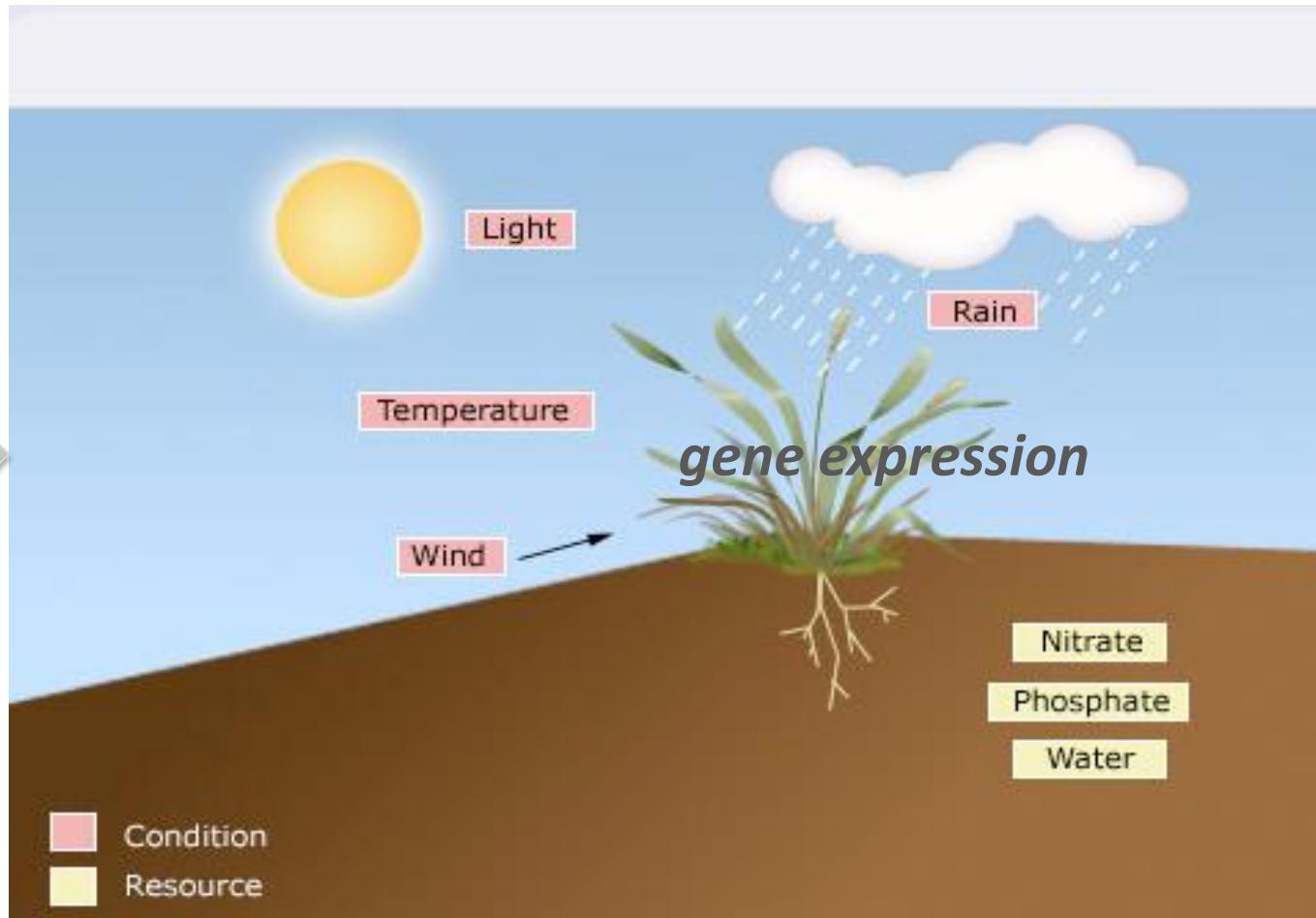
# QUALITY OF STARTING VEGETABLE MATERIAL: NATURAL VARIABILITY OF METABOLITES



# QUALITY OF STARTING VEGETABLE MATERIAL: NATURAL VARIABILITY OF METABOLITES



**environment**





## ESTRATTI DI ORIGINE VEGETALE

## HERBAL DRUG PREPARATION



# ESTRATTI DI ORIGINE VEGETALE (Herbal Drug Preparations)

Secondo la Ph.Eur.:

## **HERBAL DRUG PREPARATIONS**

### **Plantae medicinales praeparatae**

#### **DEFINITION**

Herbal drug preparations are homogeneous products obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation.

Herbal drug preparations include, for example, extracts, essential oils, expressed juices, processed exudates, and herbal drugs that have been subjected to size reduction for specific applications, for example herbal drugs cut for herbal teas or powdered for encapsulation.

Herbal teas comply with the monograph *Herbal teas (1435)*.

*NOTE:* the term *commminuted* used in European Community legislation on herbal medicinal products describes a herbal drug that has been either cut or powdered.

The term *herbal drug preparation* is synonymous with the term *herbal preparation* used in European Community legislation on herbal medicinal products.

# ESTRATTI DI ORIGINE VEGETALE (Herbal Drug Extracts)

Quindi:

Si tratta di miscele complesse la cui riproducibilità viene garantita dalla qualità del materiale vegetale di partenza (Herbal Starting Material), dal metodo di produzione, dai controlli in corso di fabbricazione.



Manufacturing  
process /IPC



# Manufacturing of a Dry Extract



Herbal Drug (Cultivation,  
Collection, Drying, Storage)

Primary Extract

Cutting,  
Extracting

Concentrated Extract

Concentrating  
(Purification\*)

Dry Extract

Drying, Milling

Standardising,  
Quantifying

Standardized, Quantified, Others, Dry  
Extract, Refined/Purified

## Extract Types of the European Pharmacopeia

### **Standardised Extracts** : All actives are known (Active markers)

“are adjusted to a defined content of one or more constituents with known therapeutic activity. This is achieved by adjustment of the extract with inert excipients or by blending batches of the extract”.

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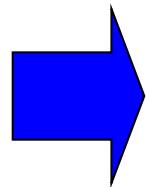
### **Quantified Extracts** : Some actives are known (Active markers)

“are adjusted to one or more active markers, the content of which is controlled within a limited, specified range. Adjustments are made by blending batches of the extract”.

---

### **Other Extracts** : None of the active is known (Analytical Markers)

“are not adjusted to a particular content of constituents. For control purposes, one or more constituents are used as analytical markers. The minimum content for these analytical markers is given in an individual monograph”.



**ESTRATTI DI  
ORIGINE VEGETALE**



**Table 1: Application of this Guide to API Manufacturing**

Type of Manufacturing	Application of this Guide to steps (shown in grey) used in this type of manufacturing				
Chemical Manufacturing	Production of the API Starting Material	Introduction of the API Starting Material into process	Production of Intermediate(s)	Isolation and purification	Physical processing, and packaging
API derived from animal sources	Collection of organ, fluid, or tissue	Cutting, mixing, and/or initial processing	Introduction of the API Starting Material into process	Isolation and purification	Physical processing, and packaging
API extracted from plant sources	Collection of plant	Cutting and initial extraction(s)	Introduction of the API Starting Material into process	Isolation and purification	Physical processing, and packaging
Herbal extracts used as API	Collection of plants	Cutting and initial extraction		Further extraction	Physical processing, and packaging
API consisting of comminuted or powdered herbs	Collection of plants and/or cultivation and harvesting	Cutting/ comminuting			Physical processing, and packaging
Biotechnology: fermentation/ cell culture	Establishment of master cell bank and working cell bank	Maintenance of working cell bank	Cell culture and/or fermentation	Isolation and purification	Physical processing, and packaging
“Classical” Fermentation to produce an API	Establishment of cell bank	Maintenance of the cell bank	Introduction of the cells into fermentation	Isolation and purification	Physical processing, and packaging

**Increasing GMP requirements**



EUROPEAN COMMISSION  
ENTERPRISE AND INDUSTRY DIRECTORATE-GENERAL

Consumer goods  
Pharmaceuticals

Brussels, 01 September 2008

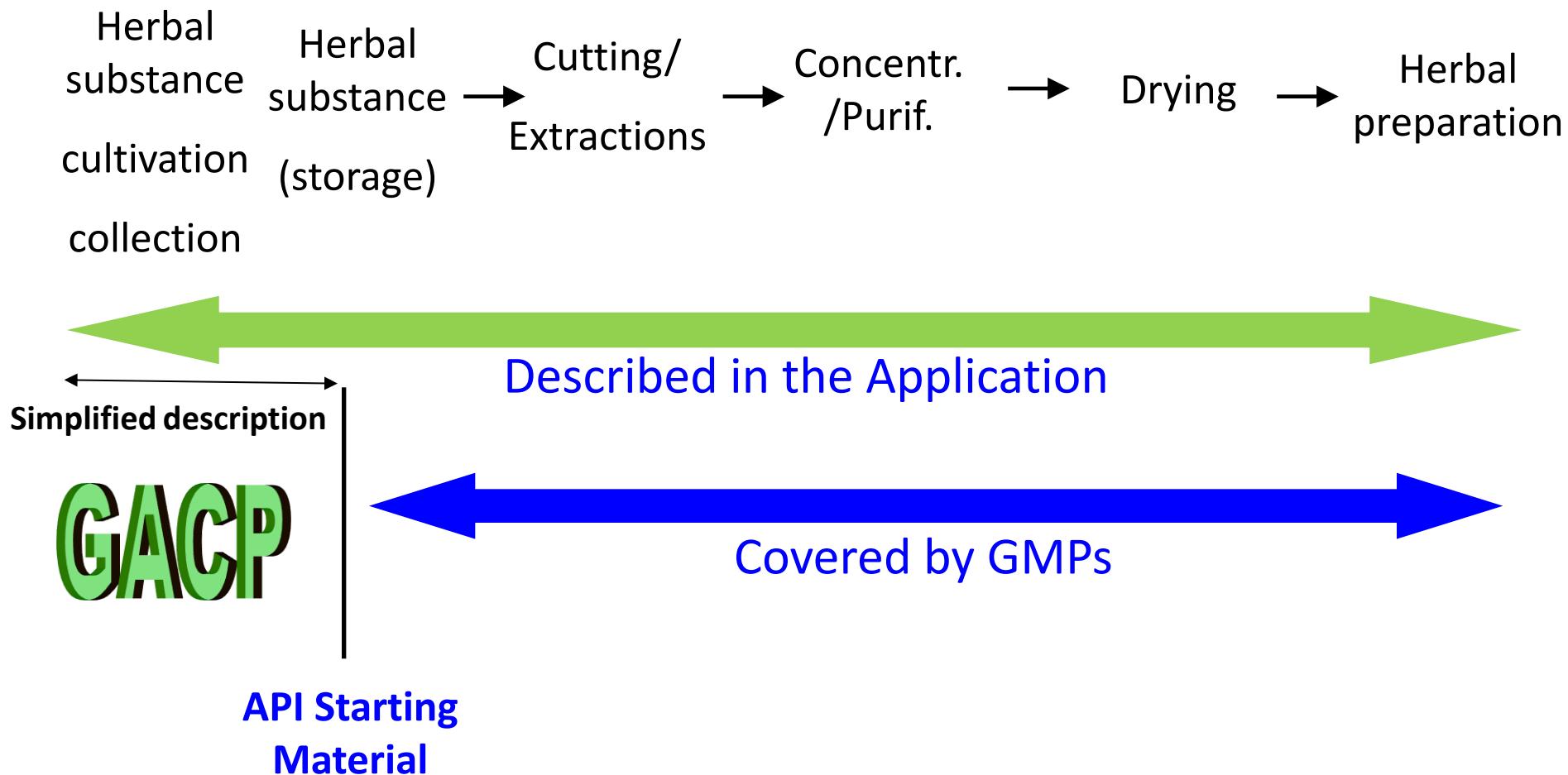
**EudraLex**  
**The Rules Governing Medicinal Products in the European Union**

**Volume 4**  
**EU Guidelines to**  
**Good Manufacturing Practice**  
**Medicinal Products for Human and Veterinary Use**

**Annex 7**  
**Manufacture of Herbal Medicinal Products**

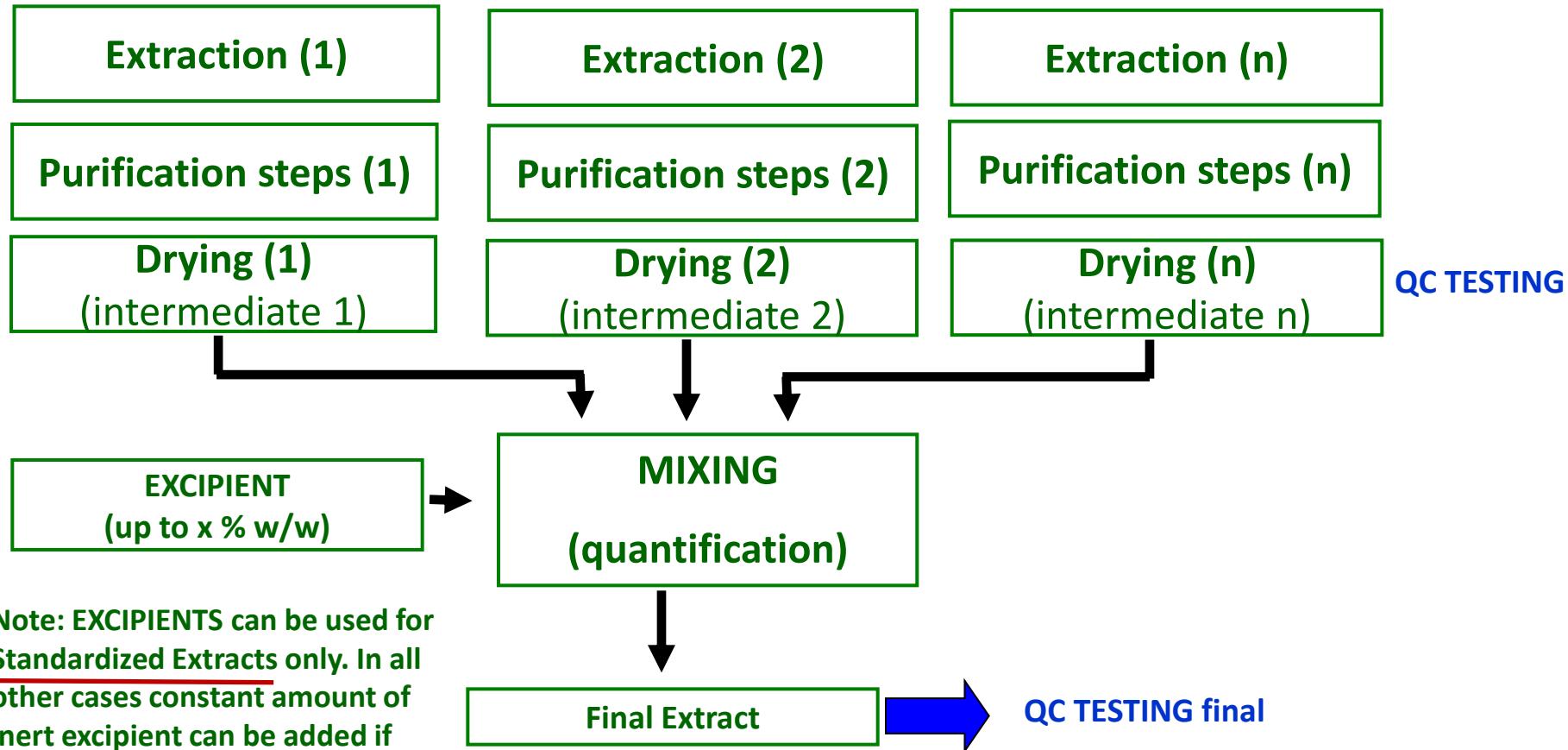
# According to current “Annex 7” EMA regulation:

## Starting Materials for Herbal extracts



# Extraction,( Purification),Drying, Standardization/Quantification

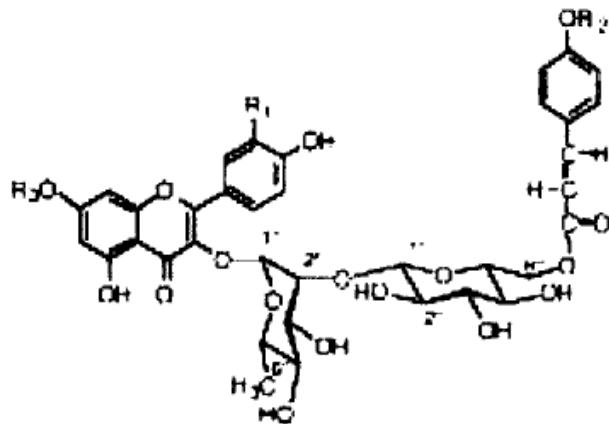
## Manufacturing Process (Ph. Eur.)





# Estratto secco dalle foglie di *Ginkgo biloba* : marker attivi

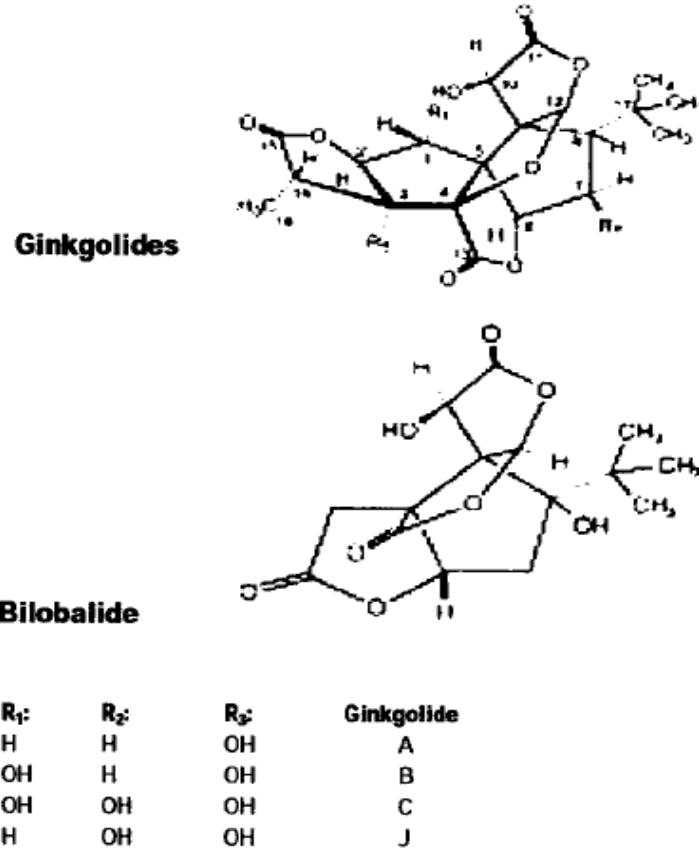
## Ginkgo flavon glicosidi (FG)



$R_1$ :	$R_2$ :	$R_3$ :	
H	H	H	3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)kaempferol
OH	glc	H	3-O-(2''-O-(6'''-O-(p-(β-D-glucosyloxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)kaempferol
OH	H	H	3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)quercetin
OH	glc	H	3-O-(2''-O-(6'''-O-(p-(β-D-glucosyloxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)quercetin
OH	H	glc	3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)-7-O-(β-D-glucosyl)quercetin

Key: glc = glucose.

## Terpenlattoni (TL)

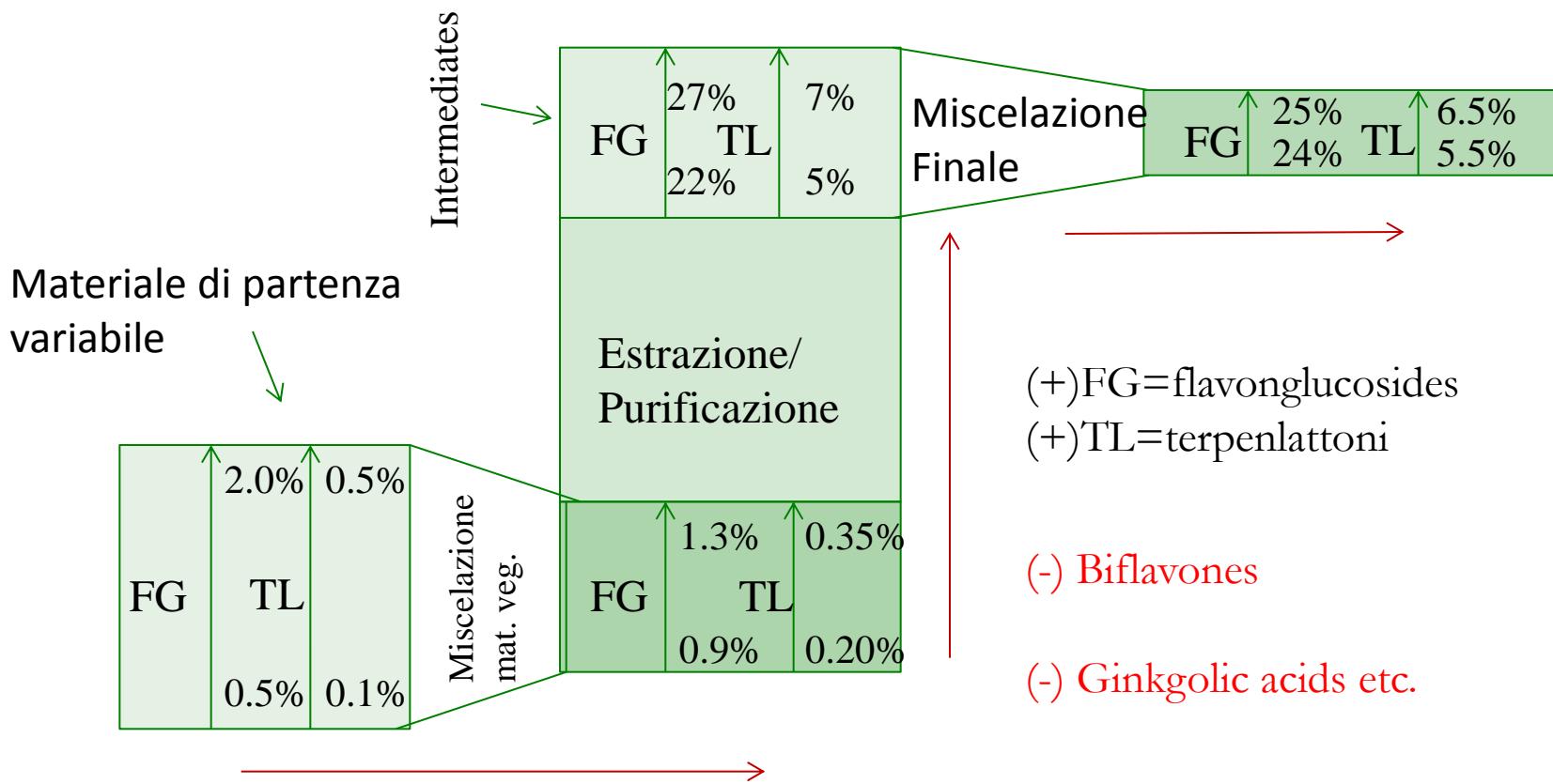


# ESTRATTI DI ORIGINE VEGETALE

## MISCELAZIONI (per compensare la variabilità naturale)

Purificazioni/quantificazione

### GINKGO BILOBA (Dry Extract, Quantified, Purified)



## ESTRATTI DI ORIGINE VEGETALE (Herbal Drug Extracts)

### Dry extracts

are solid preparations obtained by evaporation of the solvent used for their production.

Dry extracts usually have a loss on drying of not greater than 5 per cent m/m. Where justified and authorised, a loss on drying with a different limit or a test for water may be prescribed.

### Soft extracts

are semi-solid preparations obtained by evaporation or partial evaporation of the solvent used for production

# ESTRATTI DI ORIGINE VEGETALE (Herbal Drug Extracts)

European Pharmacopoeia describes additionally:

**Oleoresins**

**Liquid extraction preparations**

**Liquid (FLUID) extracts**

**Tinctures**

# Specifiche

ESTRATTI DI ORIGINE VEGETALE

HERBAL DRUG PREPARATION





EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

31 March 2011

EMA/CPMP/QWP/2820/00 Rev. 2

EMA/CVMP/815/00 Rev. 2

EMA/HMPC/162241/2005 Rev. 2

Committee for Medicinal Products for Human Use (CHMP)

Committee for Medicinal Products for Veterinary Use (CVMP)

Committee on Herbal Medicinal Products (HMPC)

Guideline on specifications: test procedures and acceptance criteria for herbal substances<sup>1</sup>, herbal preparations<sup>2</sup> and herbal medicinal products<sup>3</sup>/traditional herbal medicinal products

Final

# ESTRATTI DI ORIGINE VEGETALE

## HERBAL DRUG PREPARATION

**GUIDELINE ON SPECIFICATIONS:  
TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR HERBAL SUBSTANCES<sup>1</sup>,  
HERBAL PREPARATIONS<sup>2</sup> AND HERBAL MEDICINAL PRODUCTS<sup>3</sup>/TRADITIONAL  
HERBAL MEDICINAL PRODUCTS**

London, 30 March 2006  
CPMP/QWP/2820/00 Rev 1  
EMEA/CVMP/815/00 Rev 1

### Specification for Herbal Preparations:

- a) Definition: a statement of the botanical source, and the type of preparation (e.g. dry or liquid extract). The ratio of the herbal substance to the genuine herbal preparation must be stated.

#### Commento:

Ratio of Herbal Substance to Genuine Herbal Preparation: DER

DER=Rappresenta il rapporto fra la massa vegetale di partenza e quella dell'estratto finale (nativo) senza l'eccipiente, e considerato “1”. E' espresso come intervallo nel seguente modo: (a-b)/1 .

# Controlli tipici di un estratto (esempio di una Monografia della Farmacopea Europea, Ginkgo biloba foglie)



## GINKGO DRY EXTRACT, REFINED AND QUANTIFIED

### CHARACTERS

**Appearance:** bright yellow-brown, powder or friable mass.

### IDENTIFICATION

#### Thin-layer chromatography (2.2.27).

*Mobile phase: anhydrous formic acid R, glacial acetic acid R, water R, ethyl acetate R (7.5:7.5:17.5:67.5 V/V/V/V).*



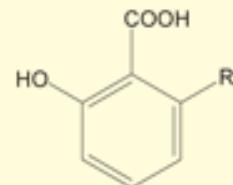
## 1. Only toxicologically relevant substances in the extract

EXAMPLE for:

### GINKGO DRY EXTRACT, REFINED AND QUANTIFIED

- *flavonoids, expressed as flavone glycosides ( $M_r$  756.7):* 22.0 per cent to 27.0 per cent (dried extract);
- *bilobalide:* 2.6 per cent to 3.2 per cent (dried extract);
- *ginkgolides A, B and C:* 2.8 per cent to 3.4 per cent (dried extract);
- *ginkgolic acids:* maximum 5 ppm (dried extract).

Assay  
Active  
markers  
  
Toxic  
(Allergenic)



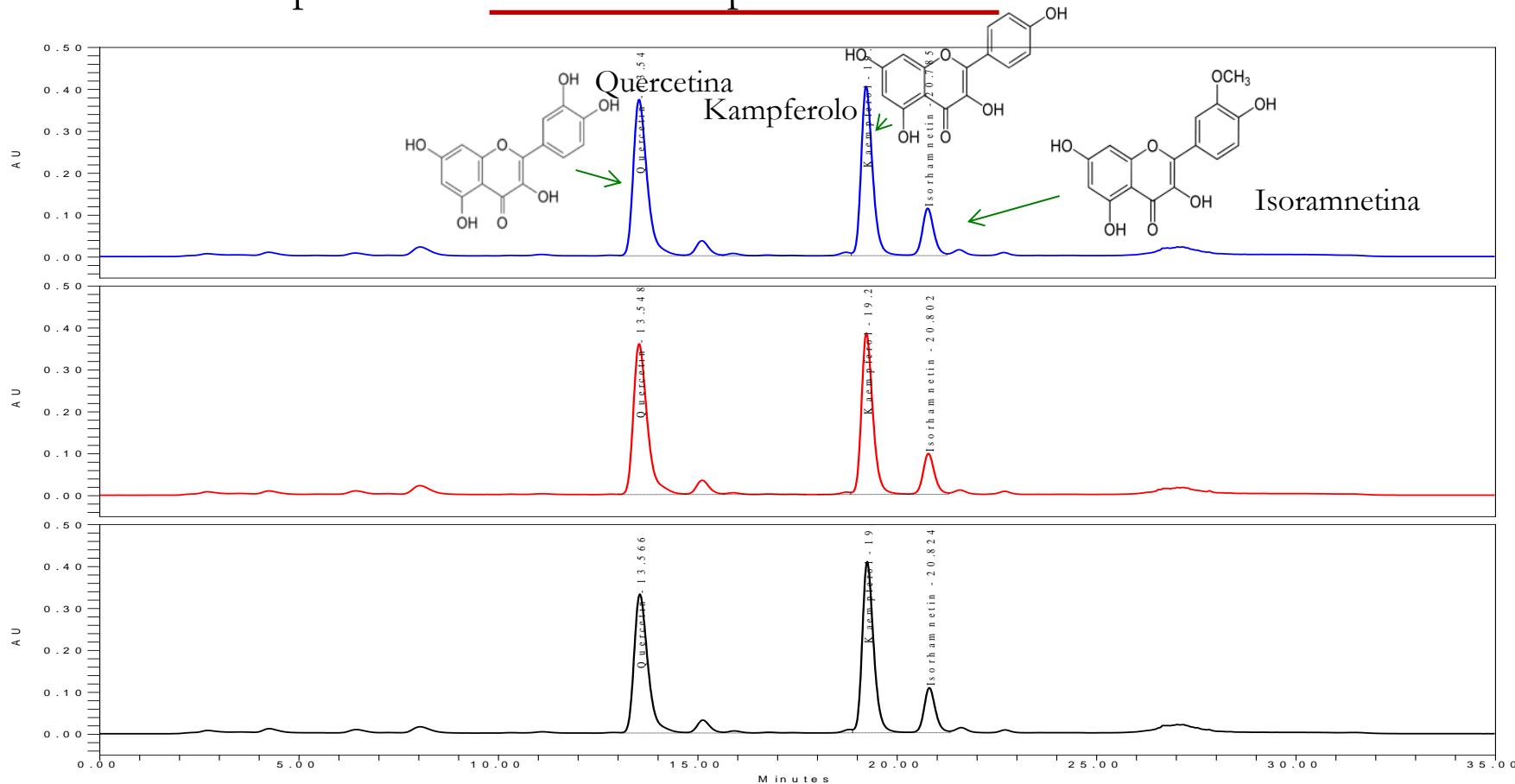
Compound	R
GA C13:0	Ginkgolic Acid C13:0 $C_{13}H_{27}$
GA C15:0	Ginkgolic Acid C15:0 $C_{15}H_{31}$
GA C15:1	Ginkgolic Acid C15:1 $C_{15}H_{29}$
GA C17:1	Ginkgolic Acid C17:1 $C_{17}H_{33}$



## Controlli tipici di un estratto (esempio di una Monografia della Farmacopea Europea, Ginkgo biloba estratto secco)

### GINKGO DRY EXTRACT, REFINED AND QUANTIFIED

Analisi del complesso dei Flavonoidi dopo idrolisi acida

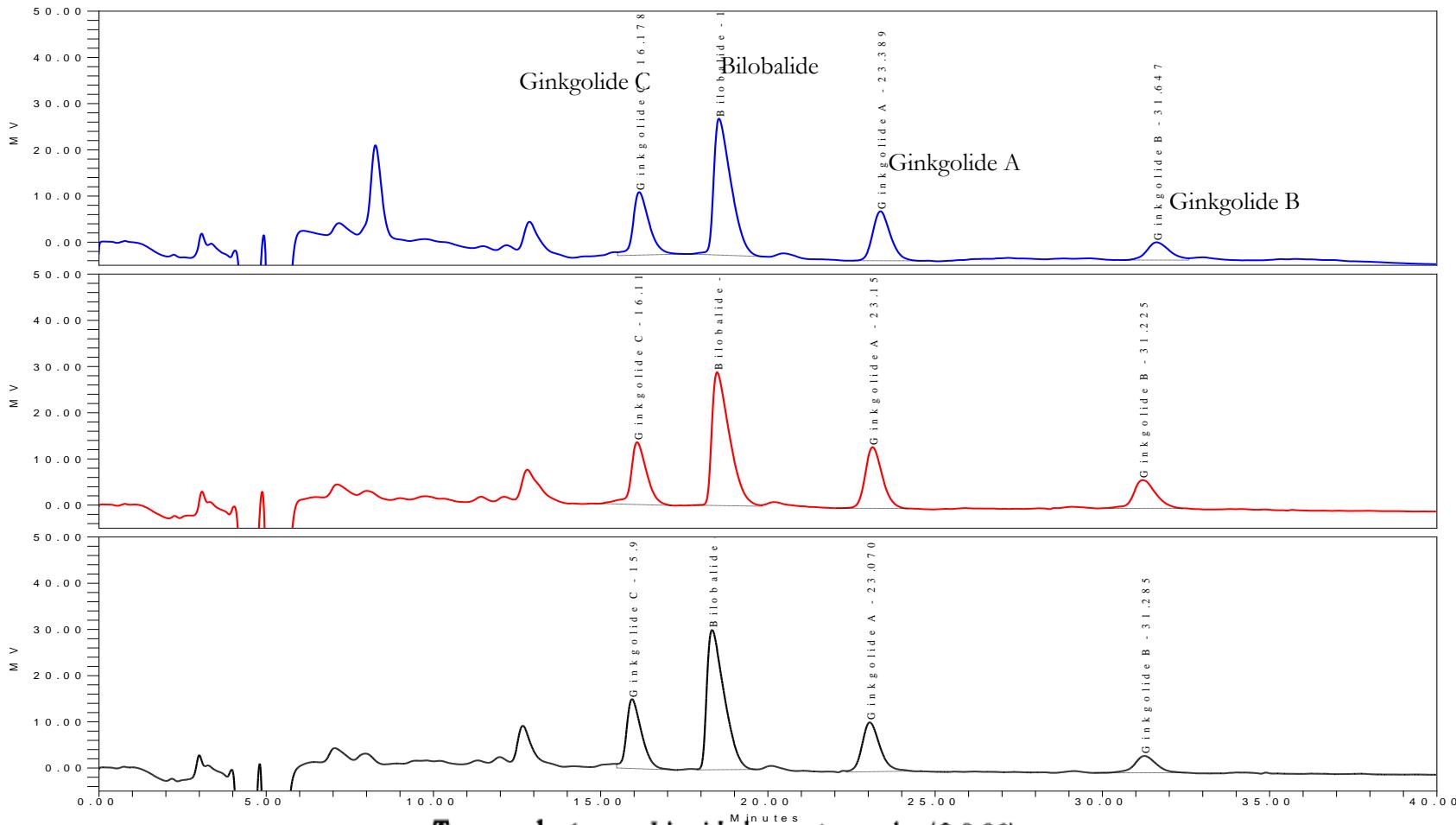


Flavonoids. Liquid chromatography (2.2.29).

Controlli tipici di un estratto (esempio di una Monografia della Farmacopea Europea, Ginkgo biloba estratto secco)



### GINKGO DRY EXTRACT, REFINED AND QUANTIFIED



Terpene lactones. Liquid chromatography (2.2.29).

## Controlli tipici di un **estratto** (esempio di una Monografia della Farmacopea Europea, Ginkgo biloba foglie)

### POTENTIAL CONTAMINANTS:

- **Residual solvents** (According to ICH: Q3C(R5);Q3C(R6); EMEA CPMP/QWP/450/03 -Rev.1 - Annex I)
- **Pesticides** (According to European Pharmacopoeia 2.8.13)
- **Heavy Metals** (According to European Pharmacopoeia)
  - Pb < 5 ppm
  - Cd < 1 ppm
  - Hg < 0.1 ppm
  - As
- **Aflatoxins/Ochratoxins** (According to European Pharmacopoeia 2.8.18 ; 2.8.22)
  - B1: < 2 ug/Kg ; sum B1 B2 G1 G2: < 4 ug/Kg
- **Pyrrolizidin Alkaloids/Tropane Alkaloids (new)**

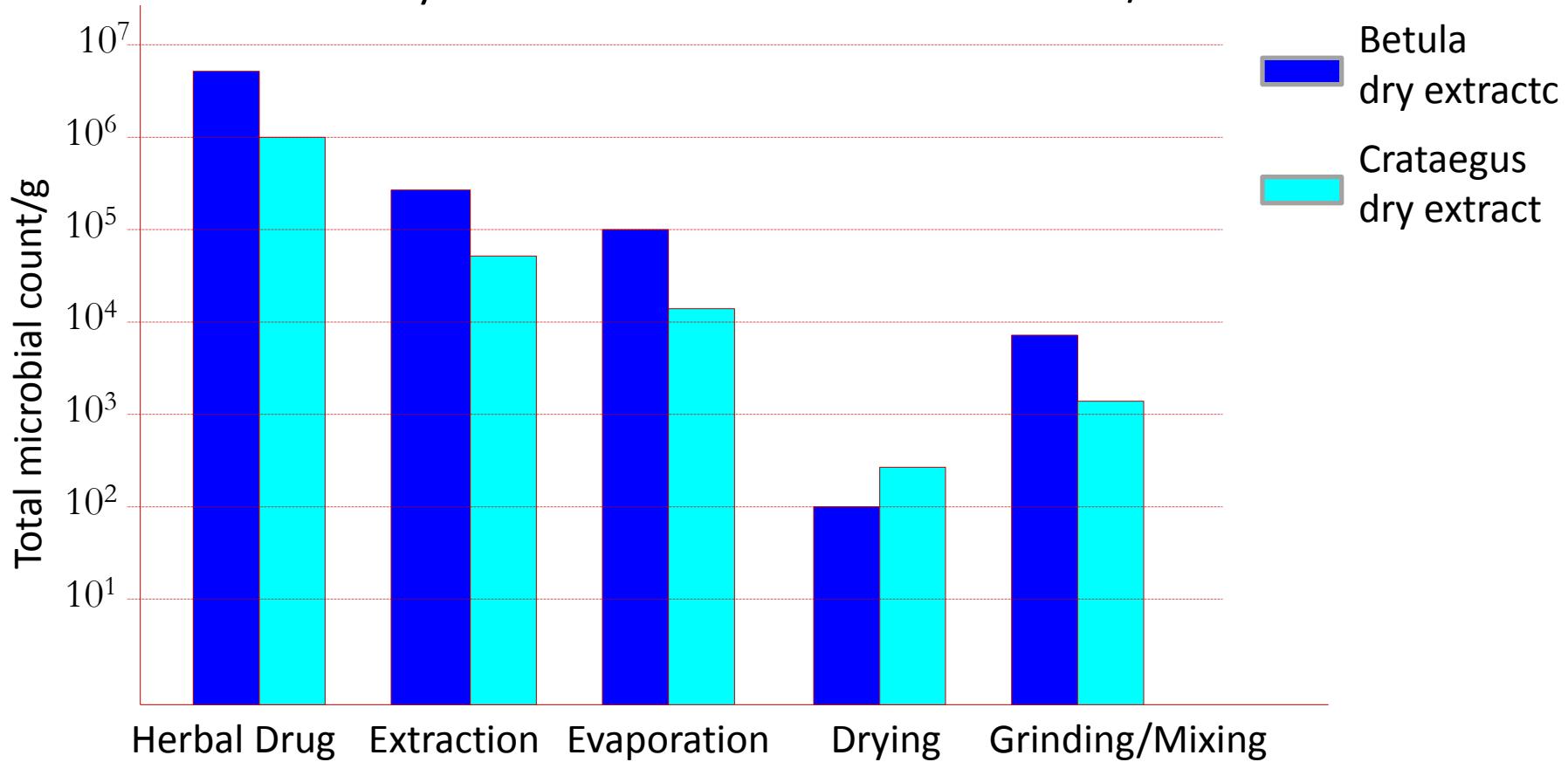


- Microbial limits: There may be a need to specify the total count of aerobic micro-organisms, the total count of yeasts and moulds, and the absence of specific objectionable bacteria. These limits should comply with those in the European Pharmacopoeia.

## ESTRATTI DI ORIGINE VEGETALE

## HERBAL DRUG PREPARATION

Example of Change of the microbial status (total microbial count/g) during the manufacture of a dry extract. Extraction solvent: Ethanol /Water



Extracted from: Herbal Medicinal Products: Gaedcke , Steinhoff, Medpharm, 2003



EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

4 June 2015  
EMA/HMPC/95714/2013  
Committee on Herbal Medicinal Products (HMPC)

# Reflection paper on microbiological aspects of herbal medicinal products and traditional herbal medicinal products

Final



European Medicines Agency  
*Post-authorisation Evaluation of Medicines for Human Use*

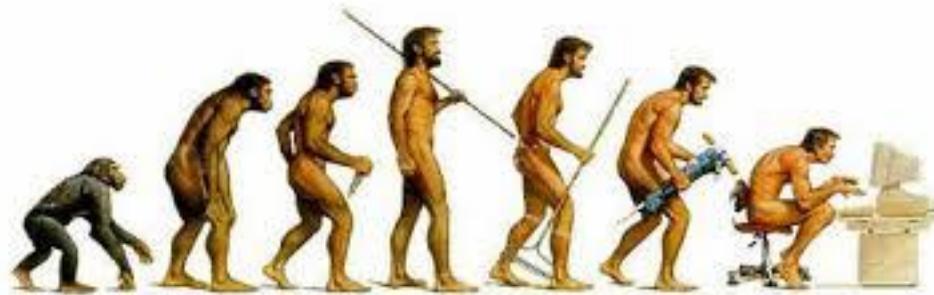
London, 21 May 2008  
Doc. Ref. EMEA/HMPC/107079/2007

**COMMITTEE ON HERBAL MEDICINAL PRODUCTS  
(HMPC)**

**GUIDELINE ON THE ASSESSMENT OF GENOTOXICITY OF  
HERBAL SUBSTANCES/PREPARIATIONS**

## ***Evolving technologies***

New analytical technology and modifications to existing technology are continuously being developed. Such technologies should be used when they are considered to offer additional assurance of quality, or are otherwise justifiable.



# HERBAL EXTRACTS

*Evolving technologies*



EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

19 September 2017

EMA/HMPC/541422/2017 Corr.<sup>1</sup>

Committee on Herbal Medicinal products (HMPC)

Concept paper on the development of a Reflection Paper  
on new analytical methods/technologies in the quality  
control of herbal medicinal products<sup>2</sup>

# **HERBAL EXTRACTS**

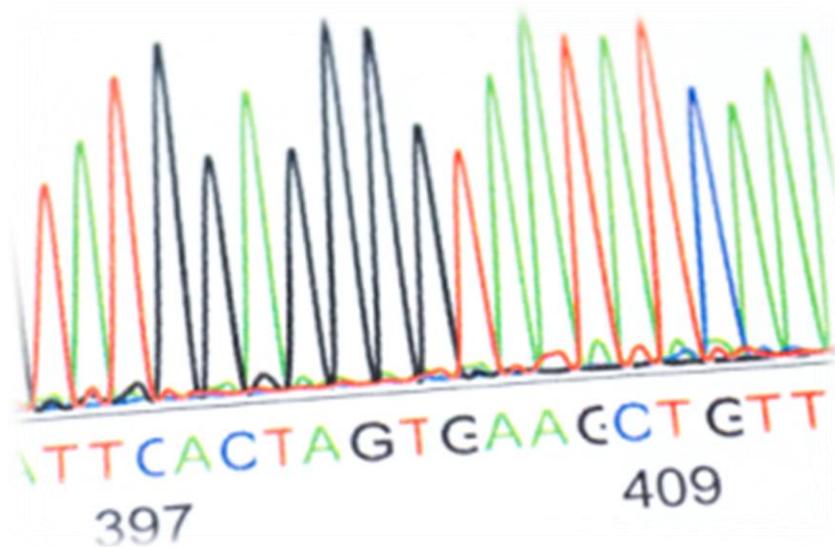
## *Evolving technologies*

- **DNA based technologies (e.g. DNA fingerprinting and DNA sequencing)**
- **Nuclear Magnetic Resonance Spectroscopy**
- **Ultraviolet (UV), mid-Infra-Red (MIR) and Near Infra-red (NIR) Spectroscopy combined with Computational Analysis**
- **Hyphenated Techniques (HPLC-MS, LC-NMR etc.)**
- **Chemometric Approaches (including Multivariate Analysis (MA) and Principal Component Analysis (PCA))**
- **Biosensors**

# STARTING HERBAL DRUG IDENTIFICATION

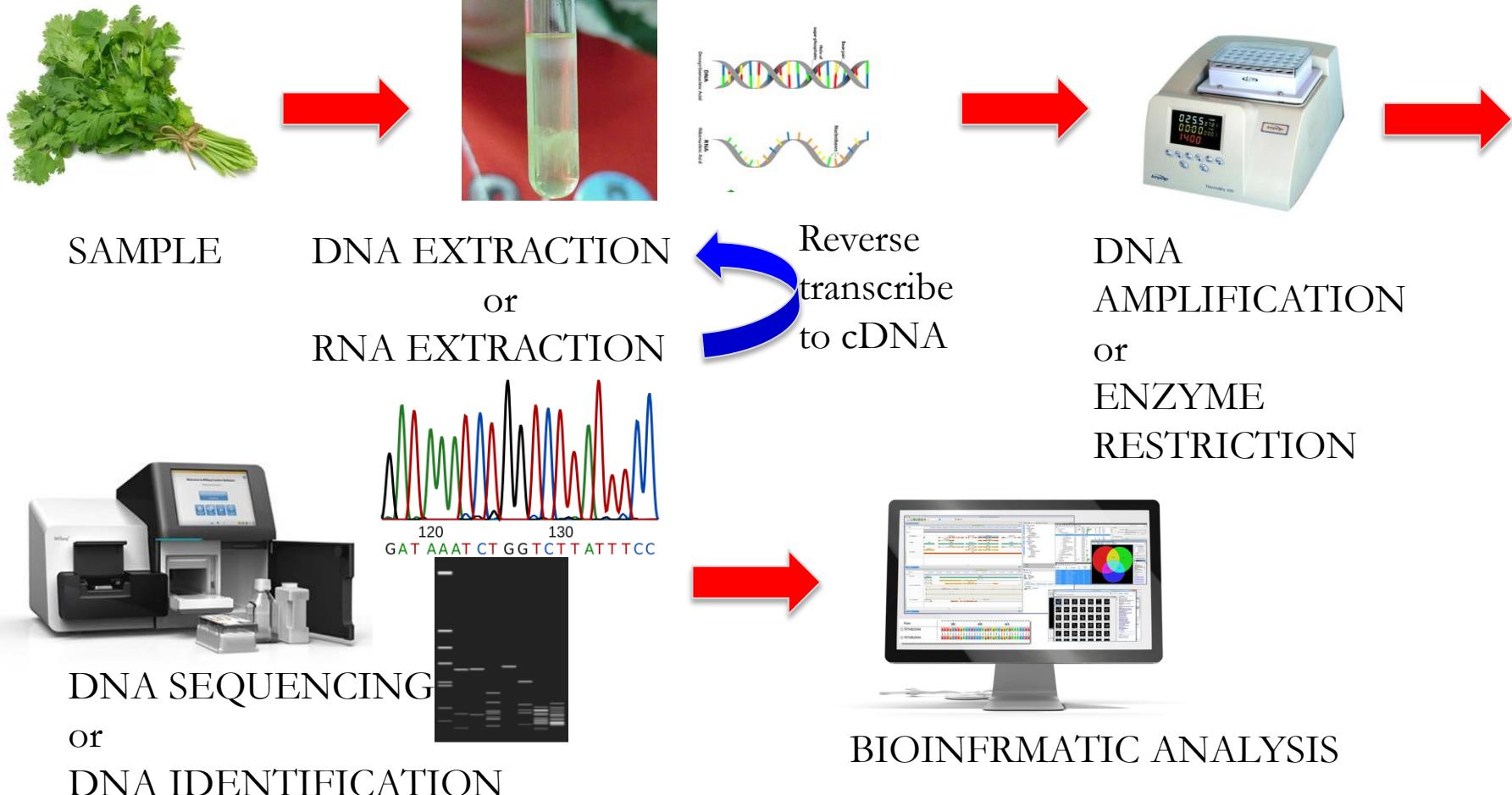
genomic approach, a new frontier

- ✓ DNA-based authentication of medicinal plants
- ✓ DNA-based authentication is a quickly, efficient, reliable and very economic (PCR)



# *Evolving technologies*

- DNA based technologies (e.g. DNA fingerprinting and DNA sequencing)



# **HERBAL EXTRACTS**

*Evolving technologies*

**Standard Analytical Controls characterize a limited number of constituents only and therefore are not appropriate for overall extract evaluation.**

**In these cases fingerprinting techniques with chromatographic or spectroscopic techniques are more suitable tools for phytoequivalence/composition studies.**

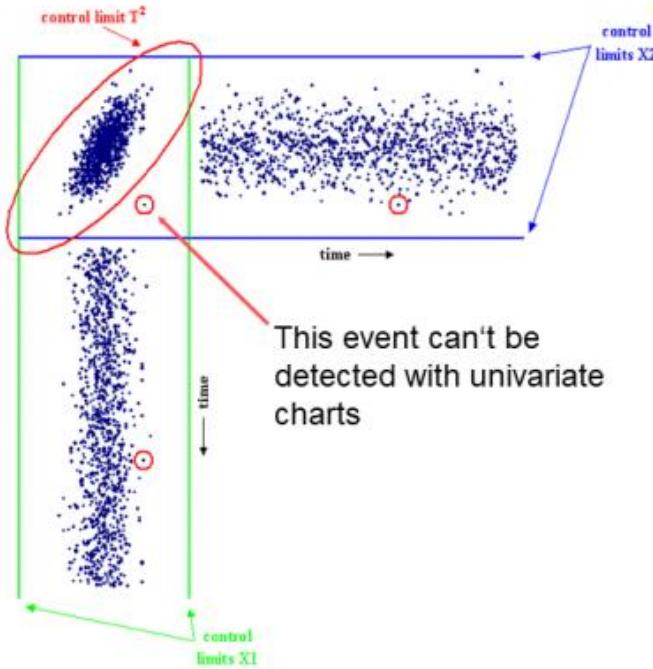
**HOW TO IDENTIFY OR COMPARE TWO EXTRACTS (SAMPLES) CONSIDERING THE OVERALL CHEMICAL COMPOSITION ?**

# HERBAL EXTRACTS

*Evolving technologies*

PCA

Move from **univariate (reductionistic)**  
to **multivariate** evaluation ! (e.g. PCA)



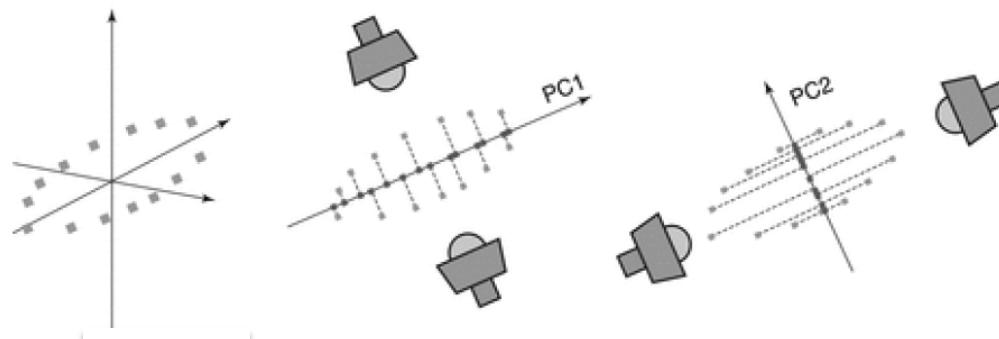
# HERBAL EXTRACTS

## *Evolving technologies*

### PCA

With PCA, we move from the one-dimensional vision of a problem to its simplified multidimensional version with a minimal loss (or improved) information .

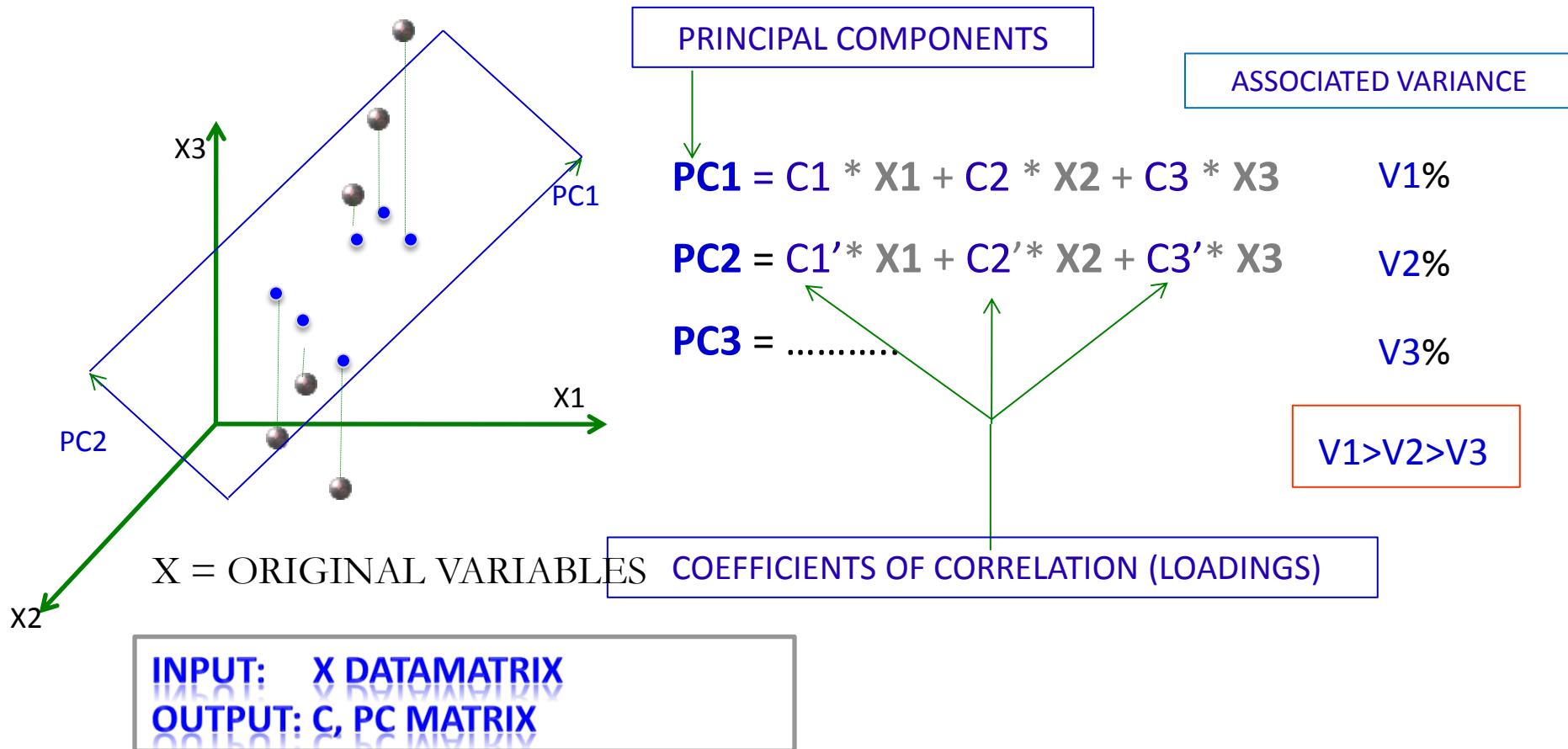
Data decomposition by projection:



Schematic representation of chemometrics.

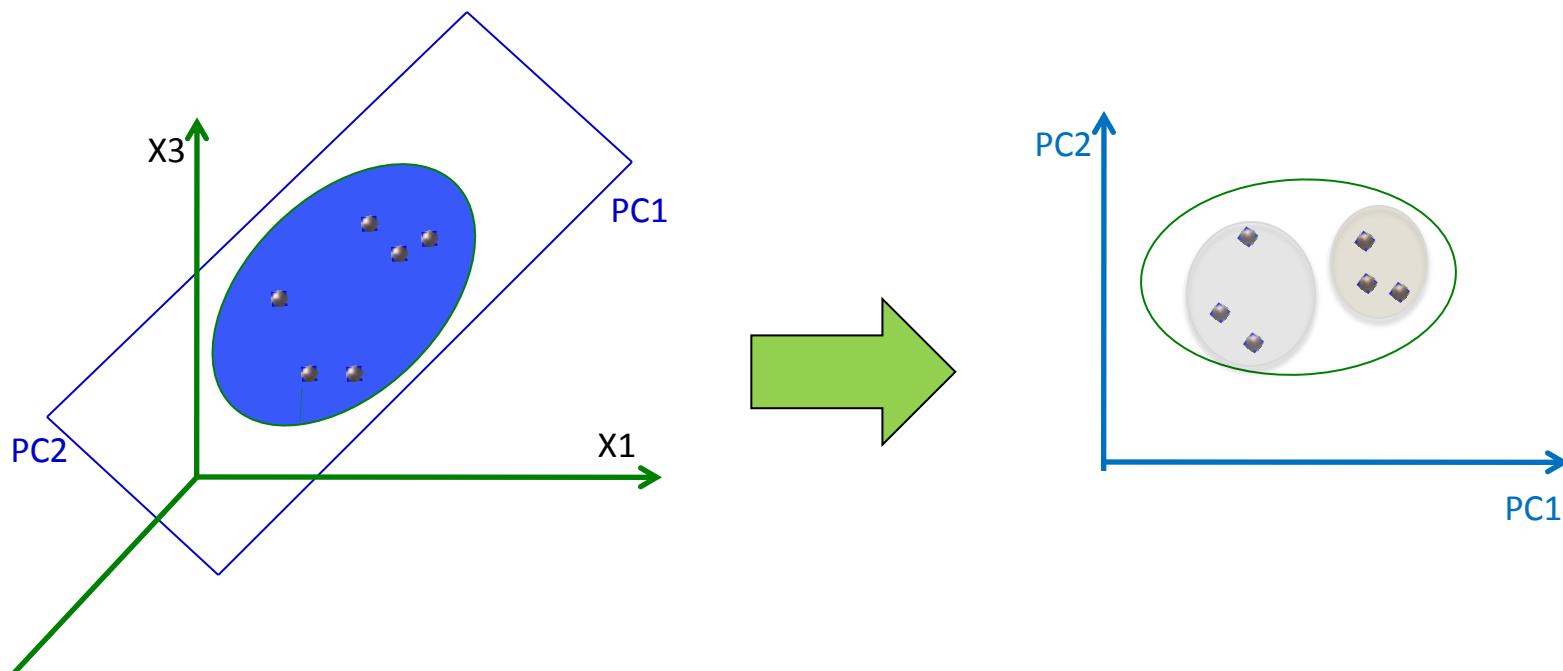
# HOW PCA WORKS

Each point can be projected down onto the directions (components pc1 e pc2) which explain the maximum of the variance so that to reduce the dimension of the ORIGINAL variables



# REDUCING THE DATA DIMENSION TO PUT INTO EVIDENCE GROUPS SEPARATION

Projection of the points down on the principal components (pc1 e pc2) which identify the **bidimensional space** where the points present the **maximum variance**.



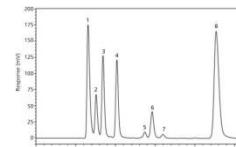
# HERBAL EXTRACTS

## *Evolving technologies*

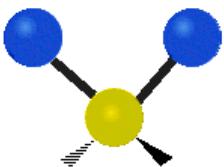
THE DATA FLOOD GENERATED BY MODERN ANALYTICAL INSTRUMENTATION PRODUCES LARGE QUANTITY OF NUMBERS THAT REPRESENT THE CHEMICAL COMPOSITION OF AN EXTRACT .:



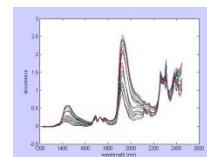
HPLC (or  
GC)



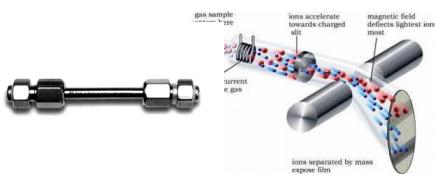
$10^1 - 10^2$  peaks



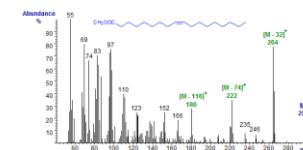
IR (FT/IR)/ UV



$10^3$  DATA POINTS



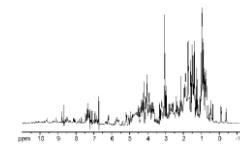
HPLC/GC-  
MS



$10^5 - 10^6$  DATA POINTS



NMR



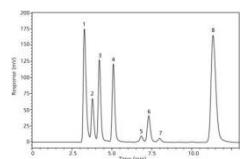
$10^4 - 10^5$  DATA POINTS

# *Evolving technologies*

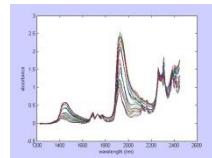
## HERBAL EXTRACTS

### TECHNOLOGY

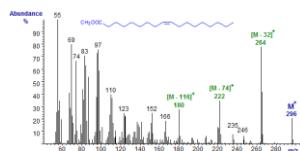
HPLC (or  
GC)



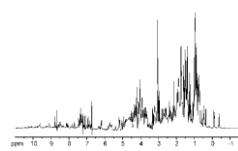
IR/NIR (FT/IR)/  
UV



HPLC/GC-  
MS



NMR



### ADVANTAGES

- Individual or Class of compounds specificity

- Overall composition detection  
Portable systems

- Individual Compound very specific /Overall composition

- Overall composition detection with good specificity

### DISADVANTAGES

- No overall composition detection

- Spectra superimposition

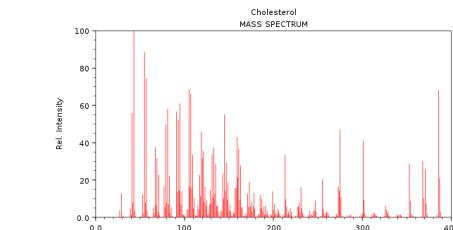
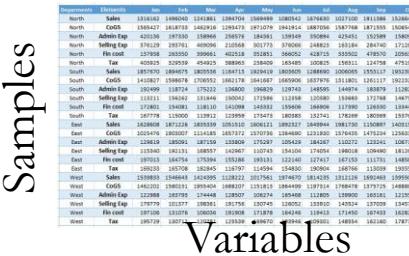
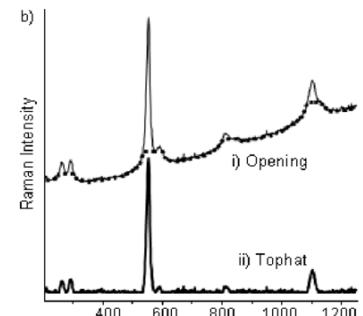
- Very high matrix dimensionality (3D)

# *Evolving technologies*

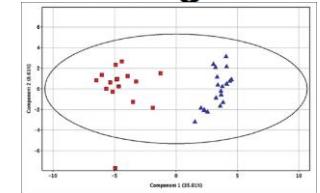
## HERBAL EXTRACTS Data preprocessing before PCA

The steps involved in the analysis of metabolomics data are at a minimum:

- **Standard post-instrument processing** of acquired spectroscopic data, such as drift/offsets baseline calculation of intensity values. e.g.: 0 baseline correction.
- Production of a **data table from the analytical measurements** such that there are  $m$  rows (observations, samples) and  $n$  columns (variables, frequencies, integrals);
- **Normalization of the data** or some related adjustment to the spectral intensities (a row operation); e.g.: normalisation to a 100 sum value (to compare different spectra for composition)
- **Scaling of the data** (a column operation); e.g. to a unit Standard Deviation
- **Multivariate statistical modeling** of the data. e.g. : PCA



$$x' = \frac{x - \bar{x}}{\sigma}$$

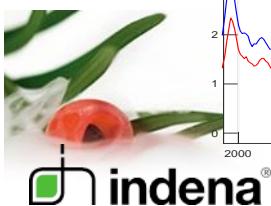
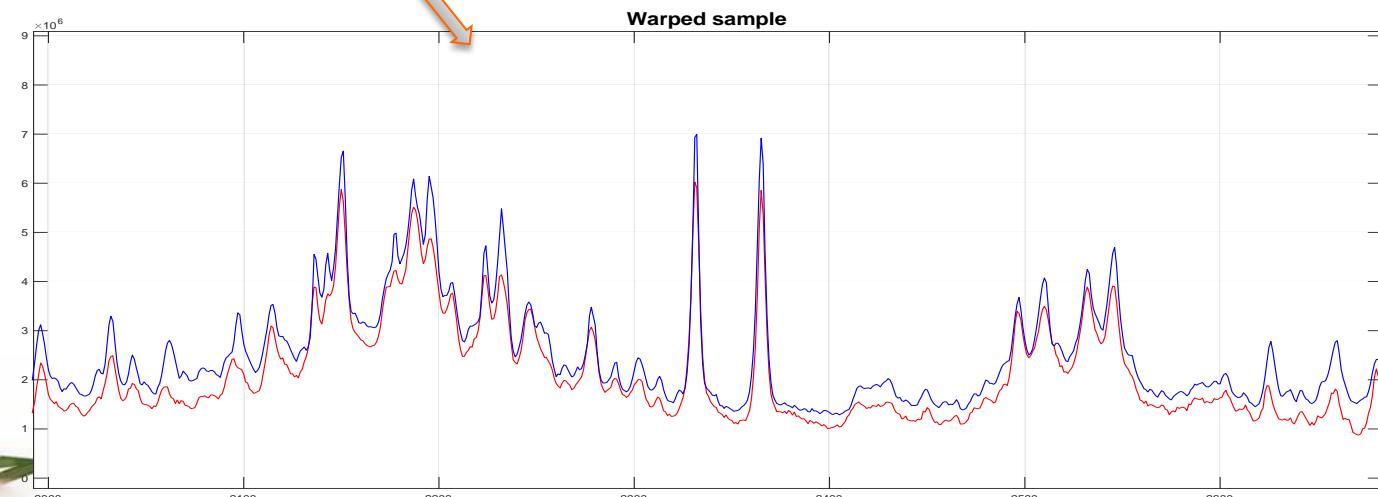
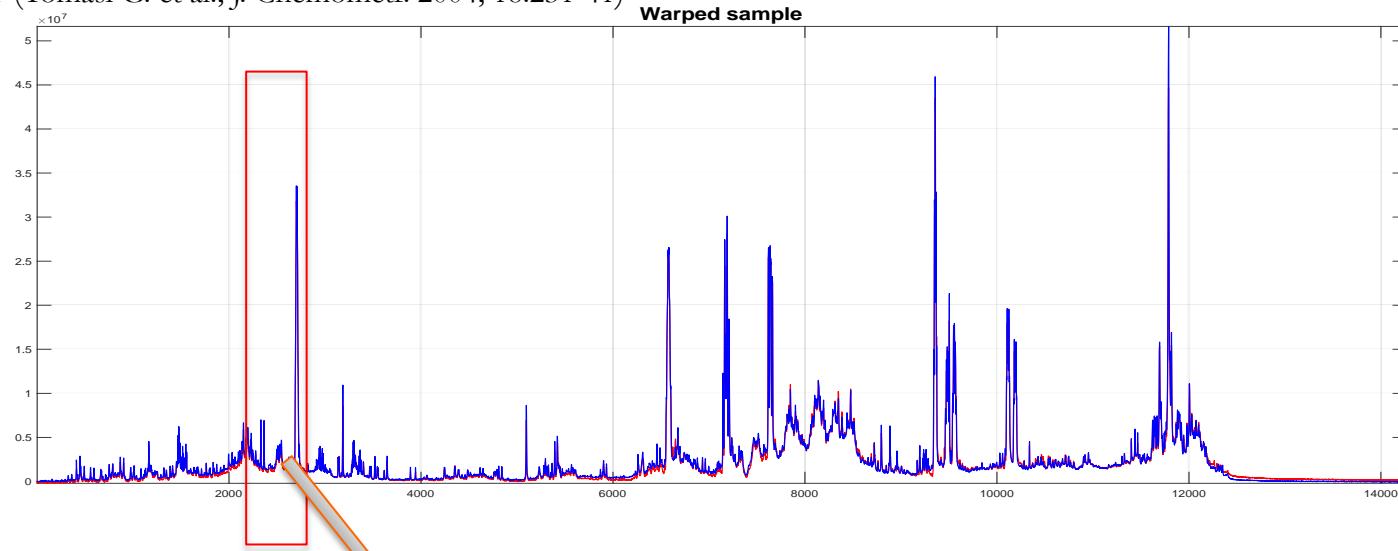


# *Evolving technologies*

## HERBAL EXTRACTS (Pre processing: Spectra Alignment; e.g. COW, ICOSSHIFT )

Ref.: COW (Nielsen NPW et al. J. Chromatogr. A 1998;805:17-35)

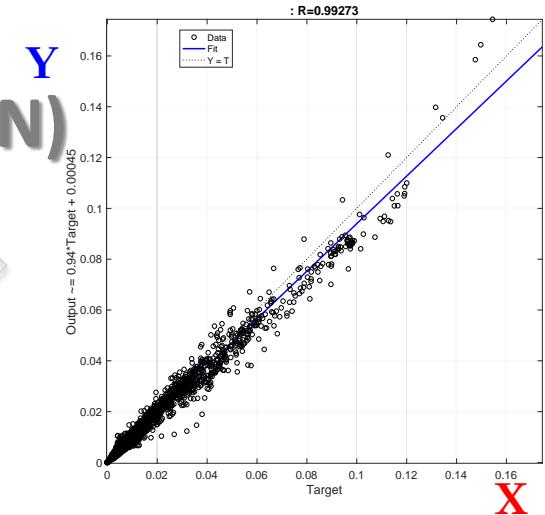
ICOSSHIFT (Tomasi G. et al.; J. Chemometr. 2004; 18:231-41)



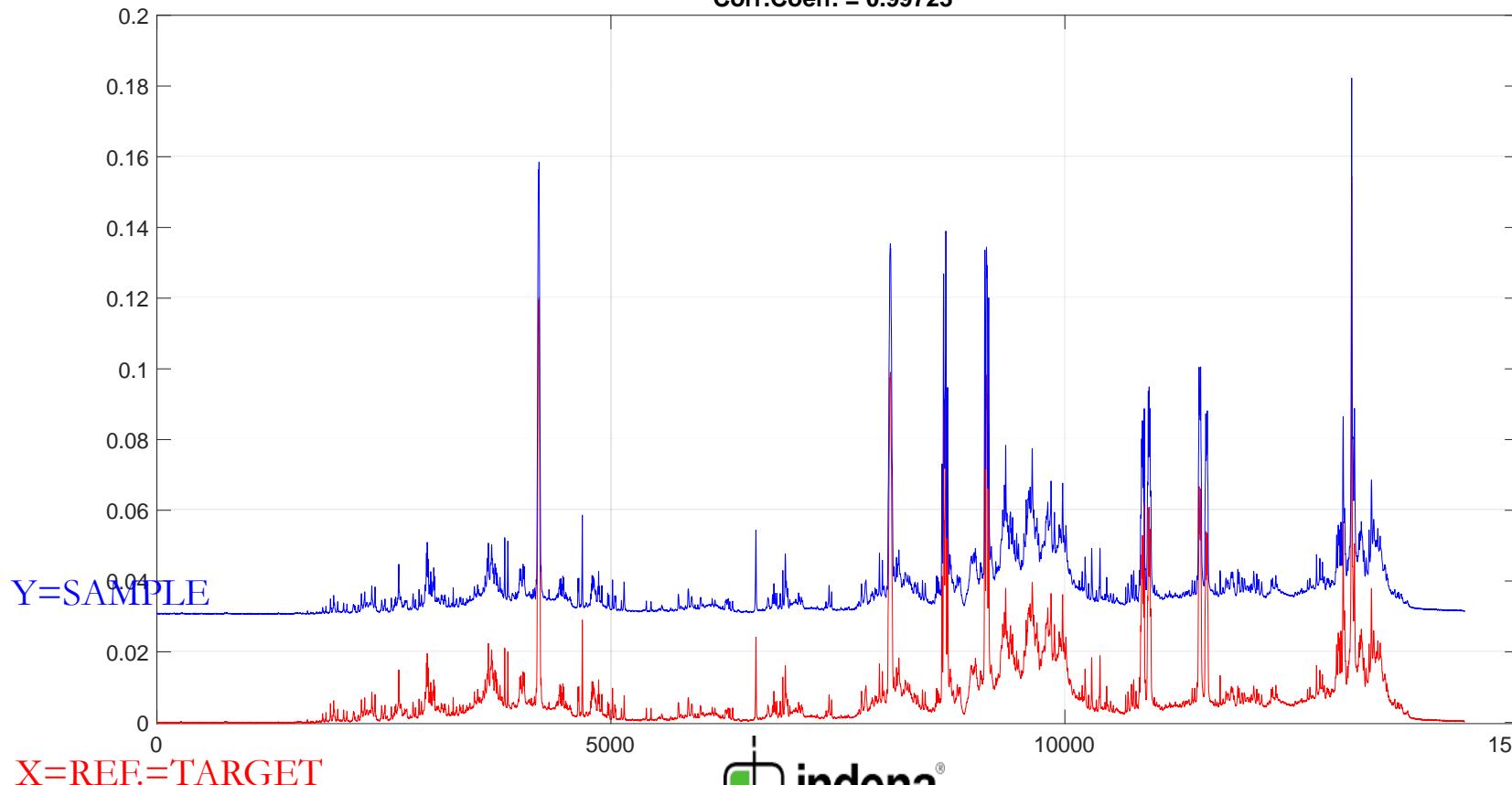
# *Evolving technologies*

## HERBAL EXTRACTS (NMR COMPARISON)

$$r = \frac{1}{n-1} \sum \left( \frac{x - \bar{x}}{s_x} \right) \left( \frac{y - \bar{y}}{s_y} \right)$$



Corr.Coeff. = 0.99723



## USE OF PCA FOR HERBAL IDENTIFICATION (SPECIES)

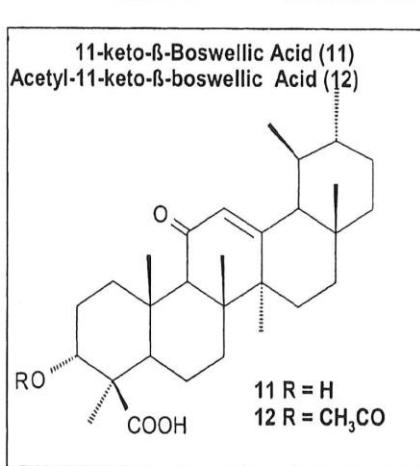
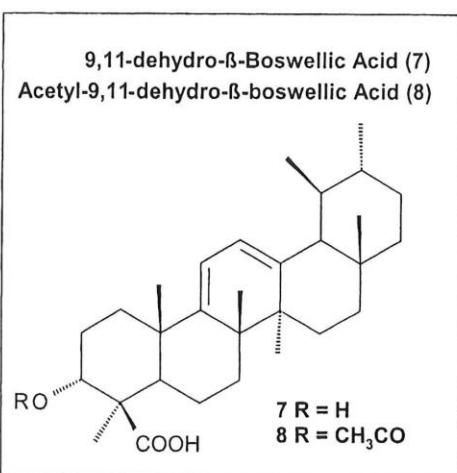
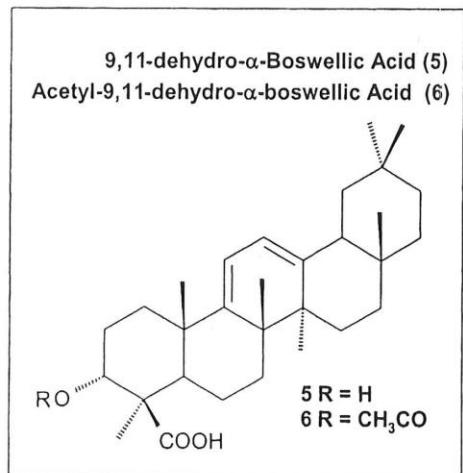
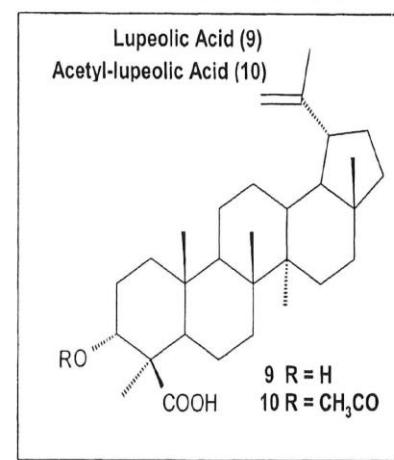
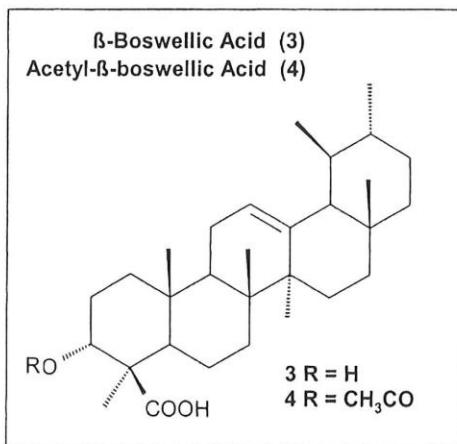
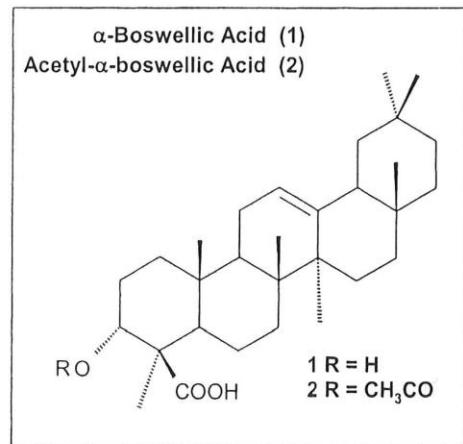
Can be used to support Herbal Identification for correct species when other methods are not suitable.

# *Evolving technologies*

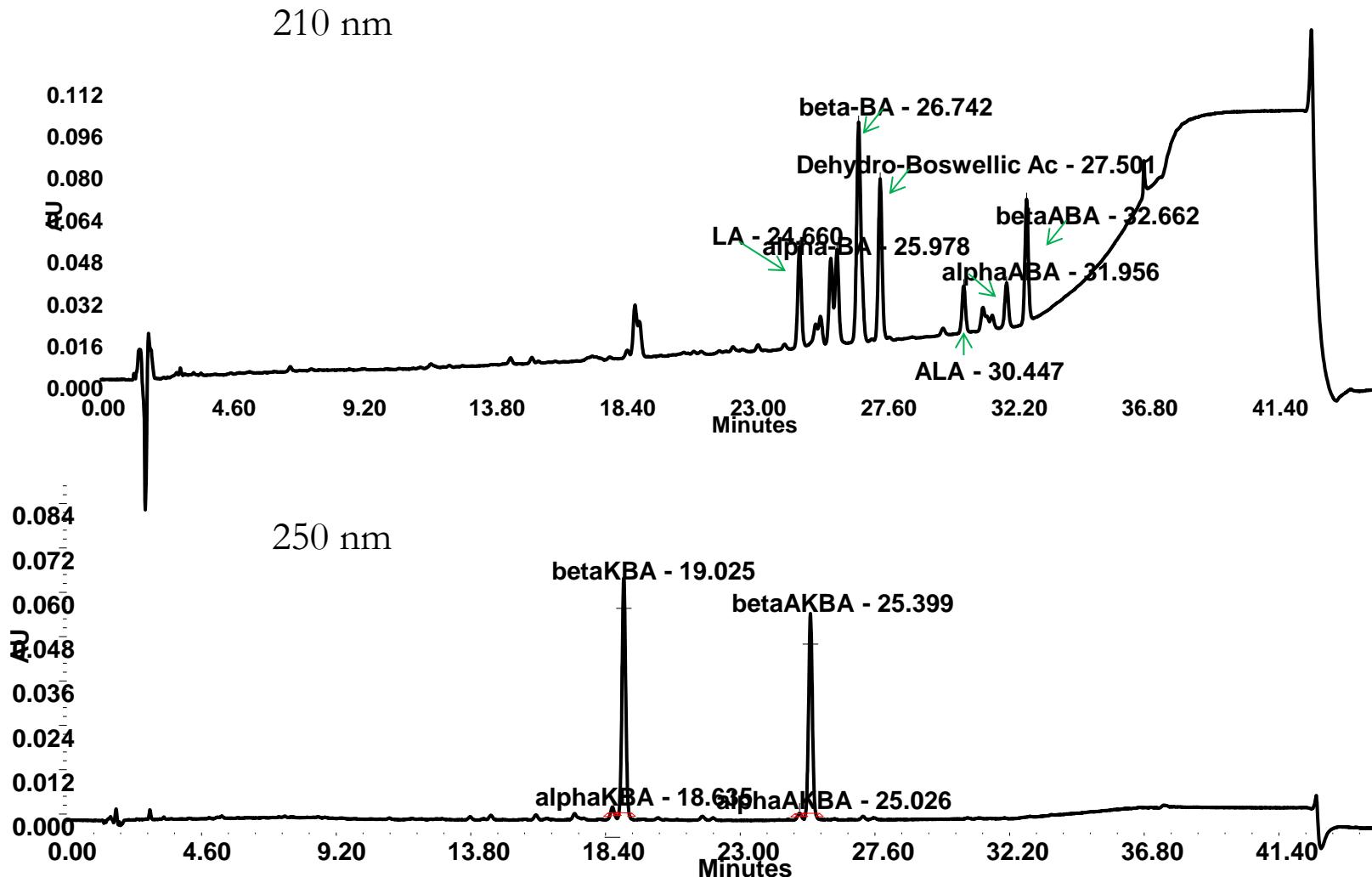
## *BOSWELLIA SERRATA RESIN*



### Boswellic acids (Active triterpenoids)

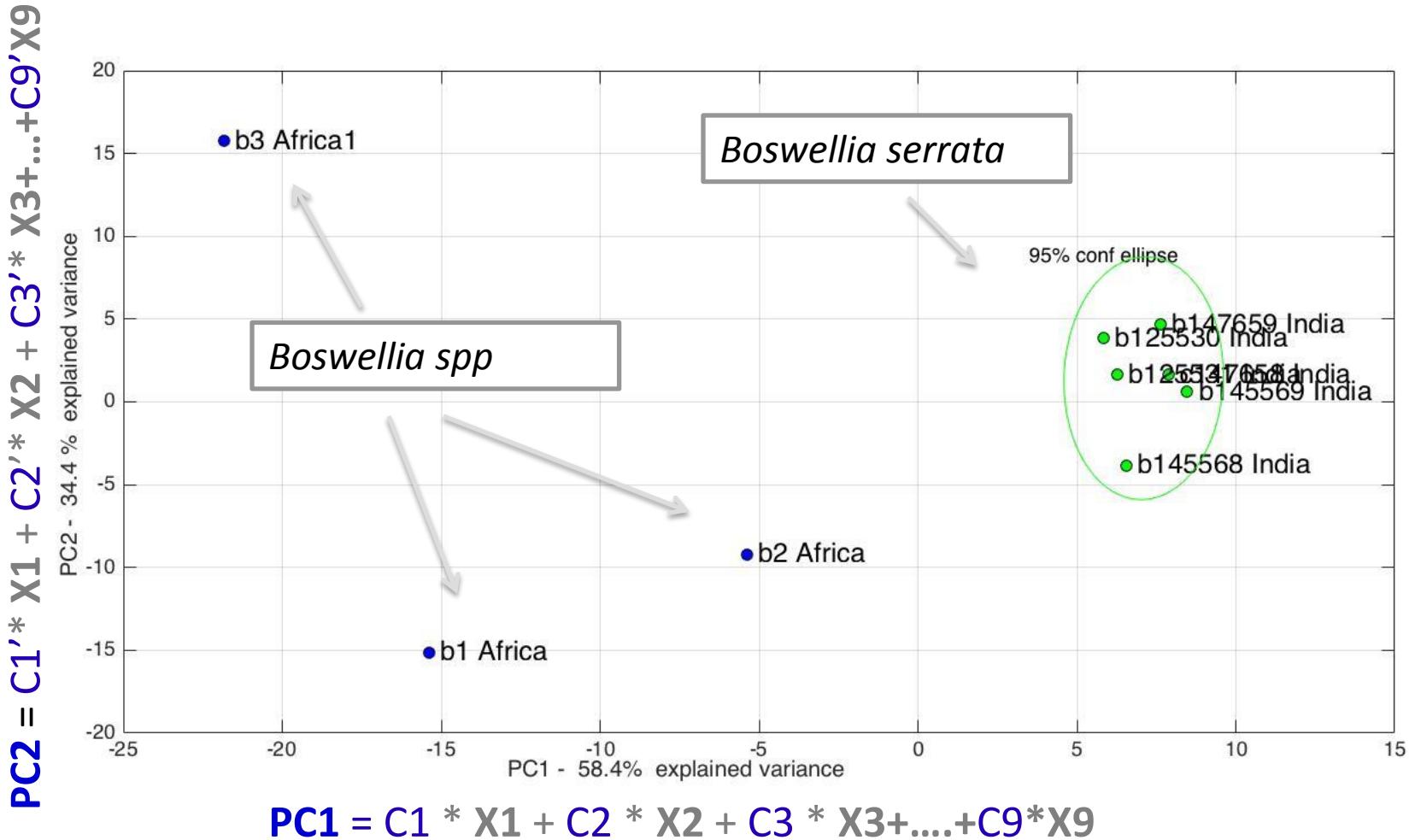


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*BOSWELLIA SERRATA TYPICAL TRITERPENOIDS (HPLC)*



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## *BOSWELLIA SERRATA RESIN*



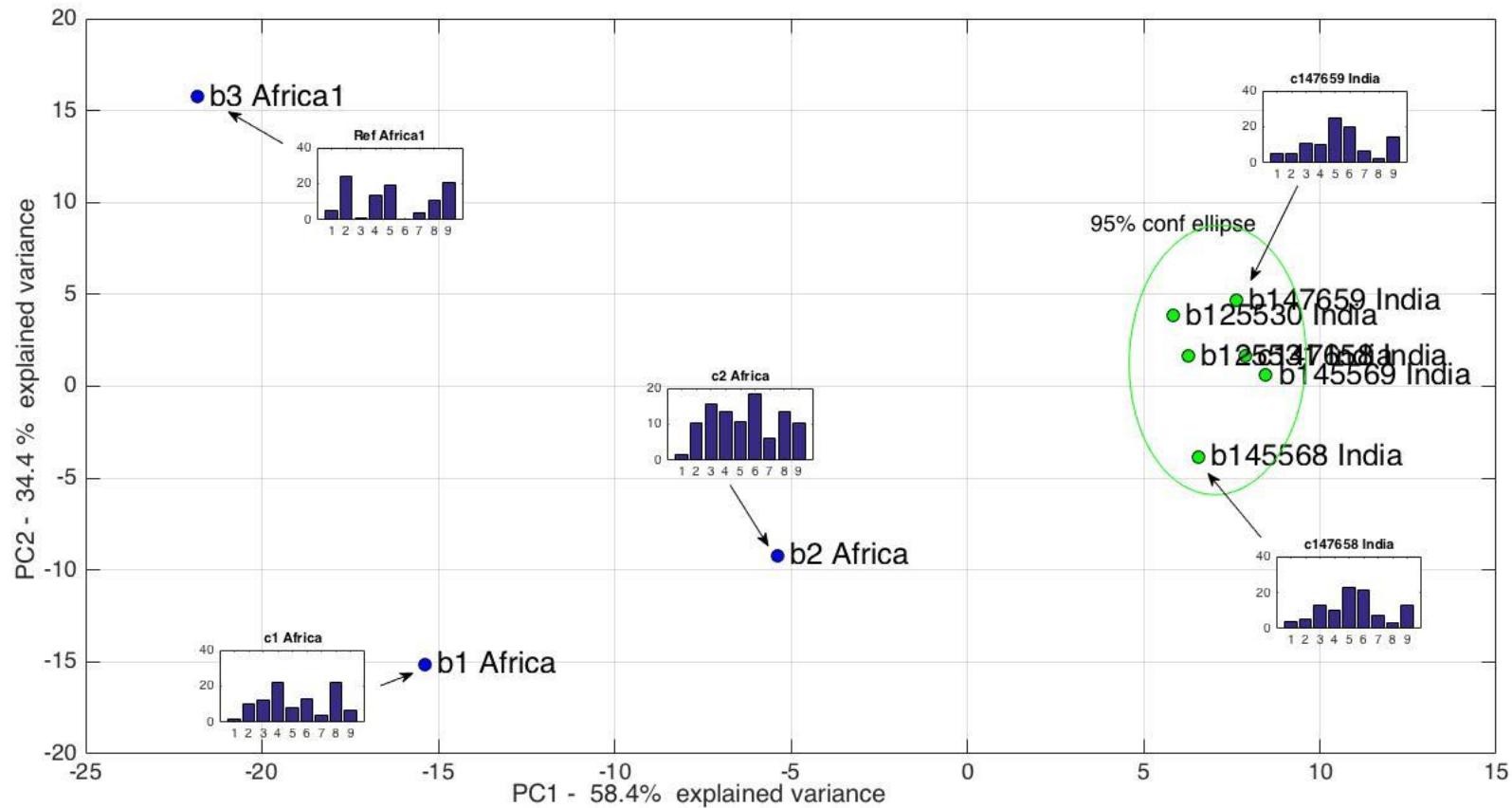
PCA of HPLC profile (HPLC peaks areas =  $\mathbf{X}$  vectors)

$C$  = loading (correlation coefficient)

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## BOSWELLIA SERRATA RESIN

$$PC2 = C1' * X1 + C2' * X2 + C3' * X3 + \dots + C9' * X9$$

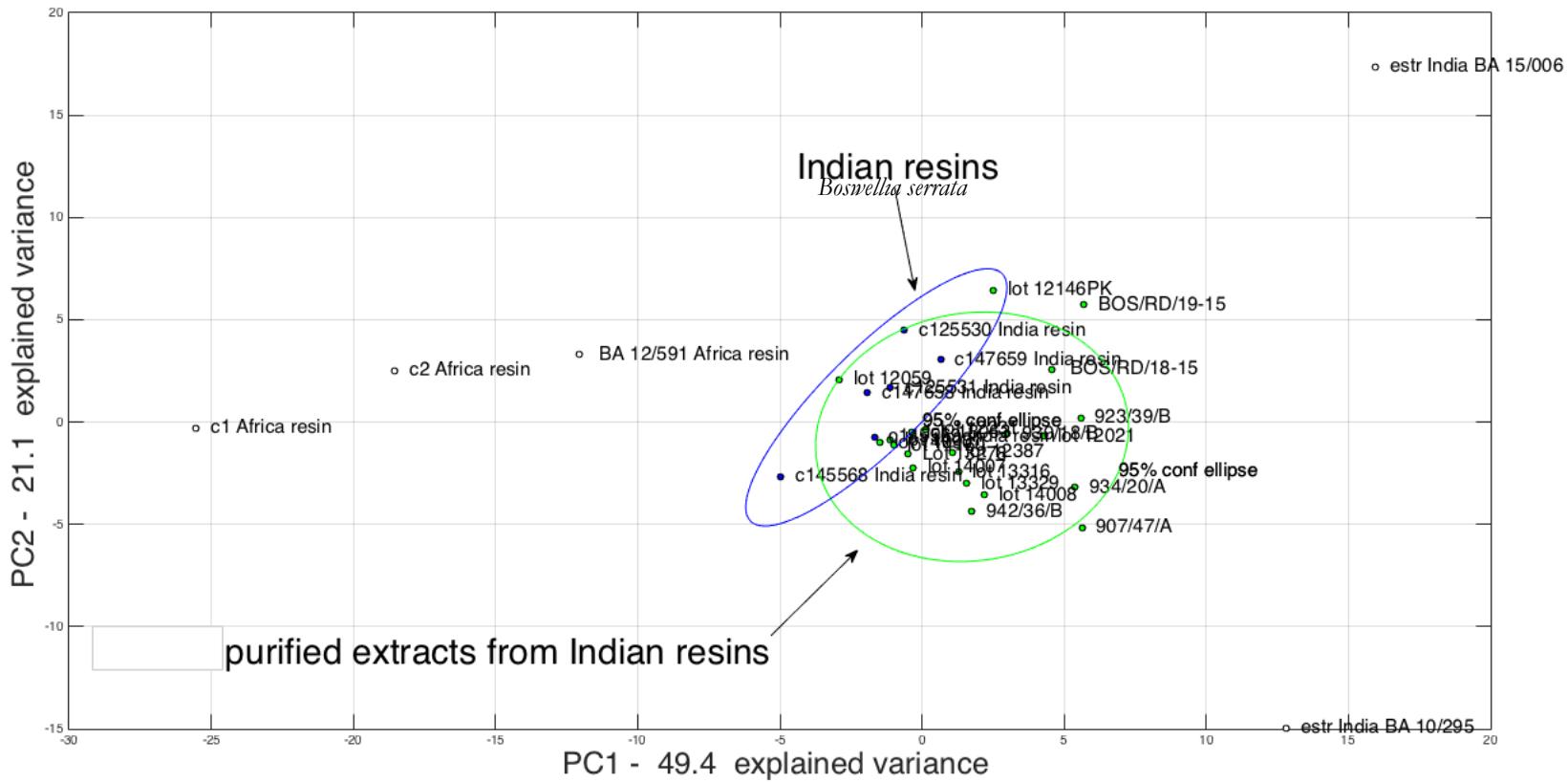


$$PC1 = C1 * X1 + C2 * X2 + C3 * X3 + \dots + C9 * X9$$

PCA of HPLC profile (HPLC peaks areas =  $\mathbf{X}$  vectors)

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## RESIN VS EXTRACTS TRITERPENE COMPOSITION



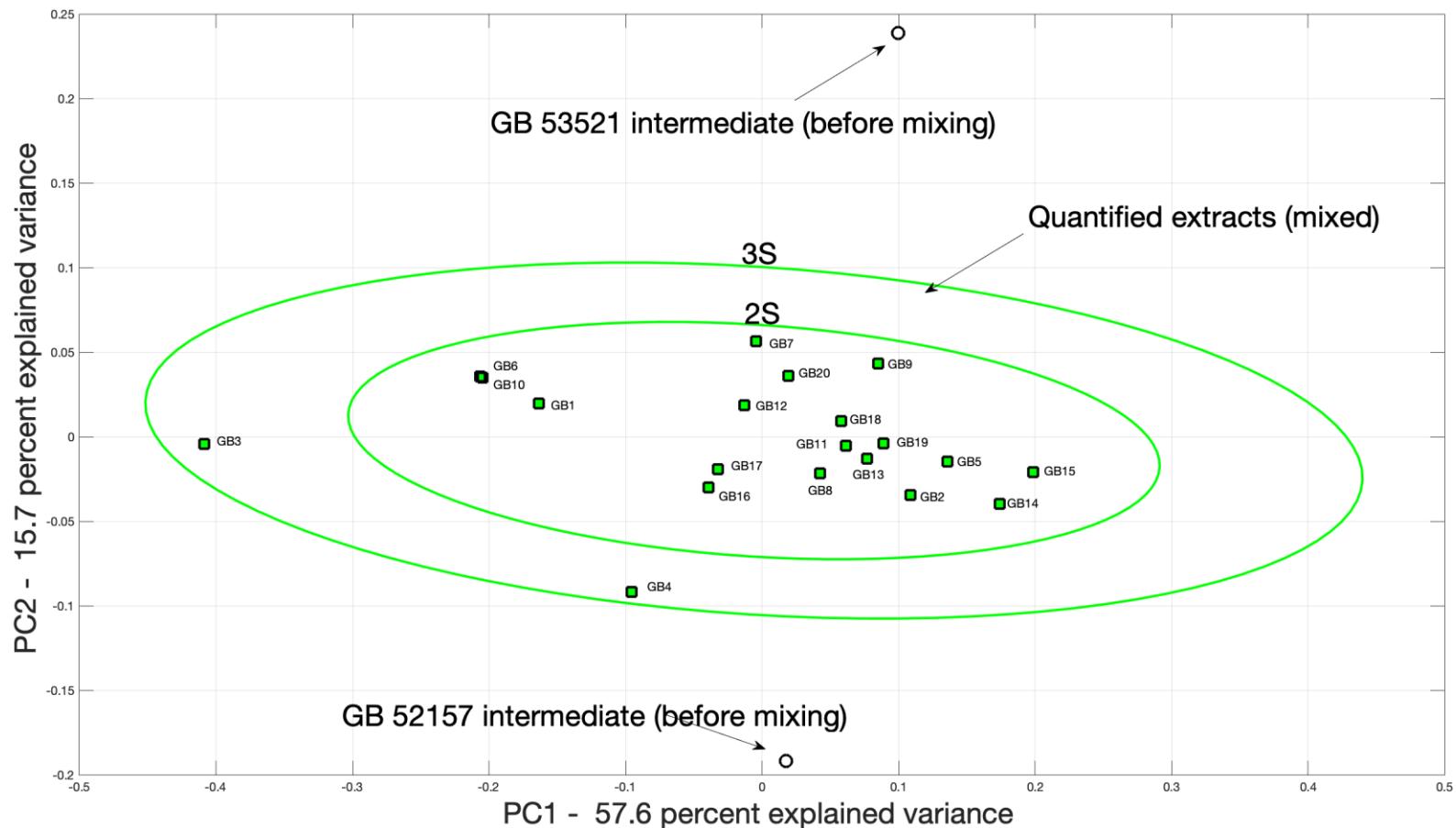
*Boswellia* resins and extracts – PCA of HPLC triterpenic acids percent composition

## PCA AS TOOL FOR OVERALL HERBAL EXTRACTS VARIABILITY EVALUATION

Can be used as a powerful QUALITY CONTROL CHART to support the overall consistency of the composition of the extract.

# ESTRATTI DI ORIGINE VEGETALE

## GINKGO BILOBA (Dry Extracts, Quantified, Purified) PCA of NMR data



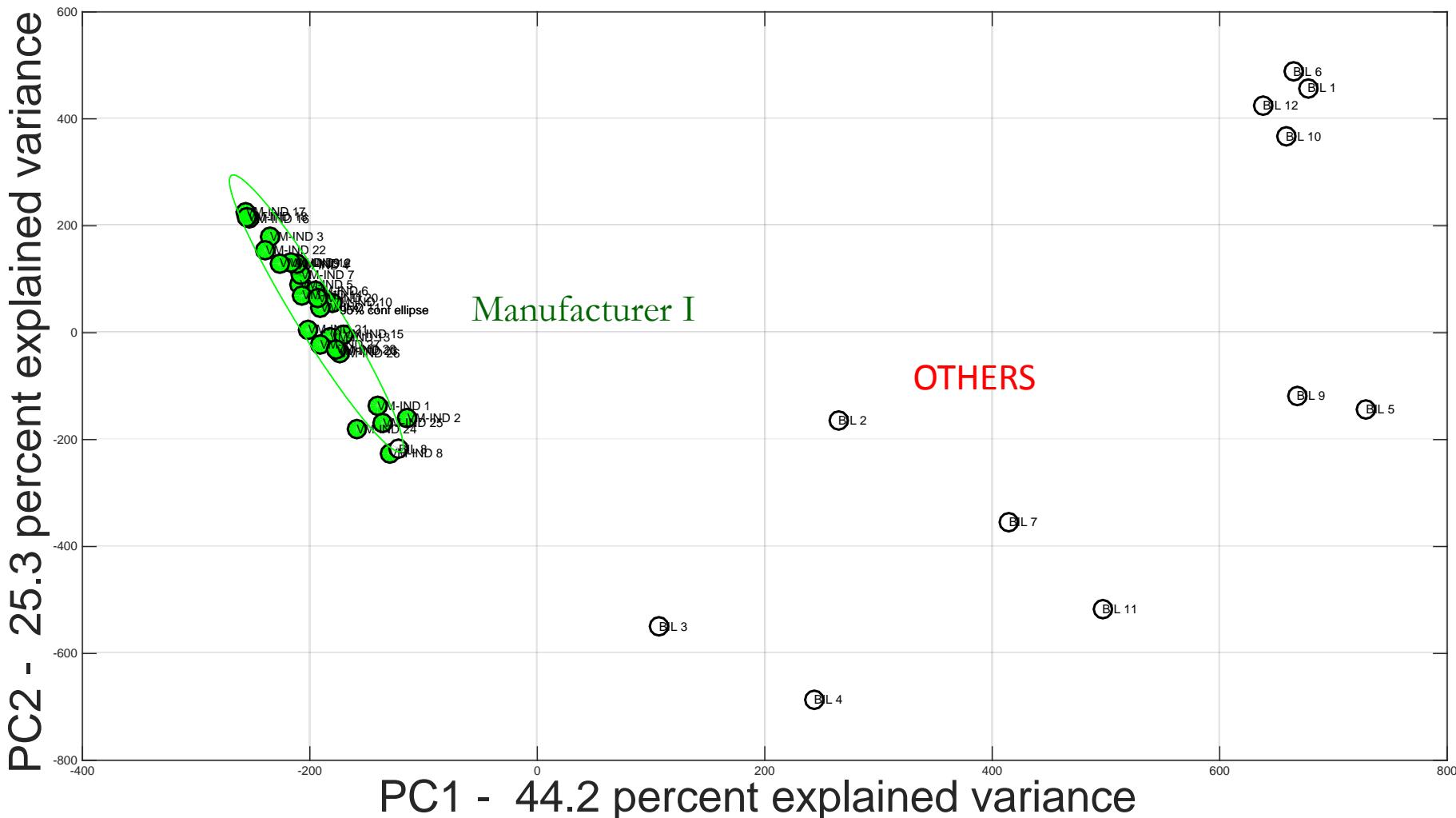
## PCA AS TOOL FOR OVERALL HERBAL EXTRACTS VARIABILITY EVALUATION

Can be used to compare extracts of different origin  
(e.g. for regulatory purposes)

# PCA AS TOOL FOR OVERALL HERBAL EXTRACTS

## VARIABILITY EVALUATION

PCA of  $^1\text{H-NMR}$  spectra of *Vaccinium myrtillus* Dry Extracts



## USE OF PCA FOR HERBAL EXTRACTS

Can be used as a powerful tool to prove/support overall phytoequivalence of two extracts (e.g. for regulatory purposes)

# HERBAL DRUG EXTRACTS

## OTHER EXTRACTS

### SERENOA REPENS (W. BARTRAM) SMALL, OILY EXTRACT



TOTAL FATTY ACIDS:

MINIMUM 80.0 PER CENT (ANHYDROUS EXTRACT)

LAURIC ACID:

MINIMUM 23.0 PER CENT (ANHYDROUS EXTRACT)

TOTAL STEROLS, EXPRESSED AS B-SITOSTEROL:

MINIMUM 0.20 PER CENT (ANHYDROUS EXTRACT)

B-SITOSTEROL:

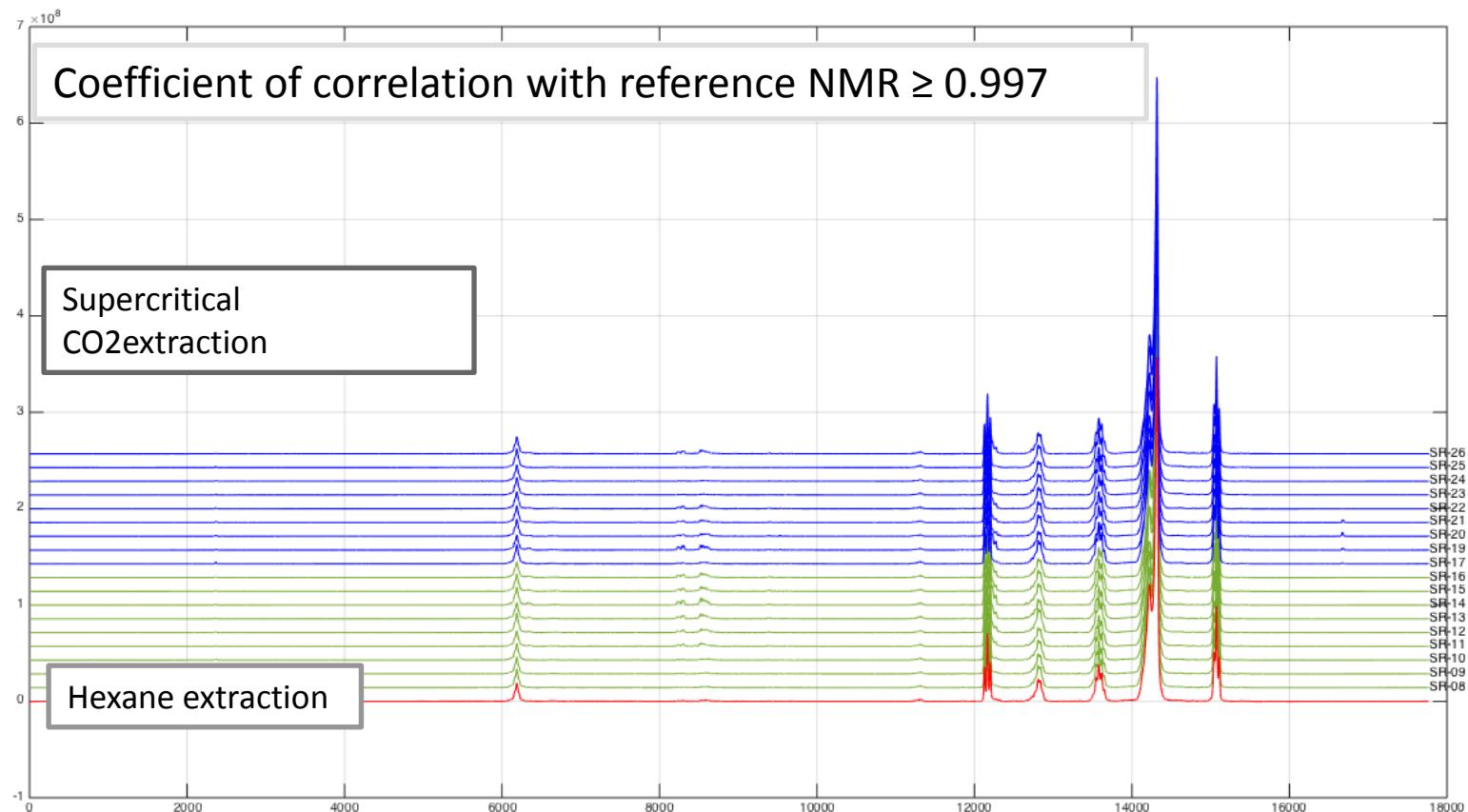
MINIMUM 0.10 PER CENT (ANHYDROUS EXTRACT)

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## PRINCIPAL COMPONENT ANALYSIS (PCA)

SERENOA REPENS EXTRACT (CO<sub>2</sub> SUPERCRITICAL AND  
HEXANE EXTRACTS PHYTOEQUIVALENCE VERIFICATION)

### NMR FINGERPRINTING (QC)

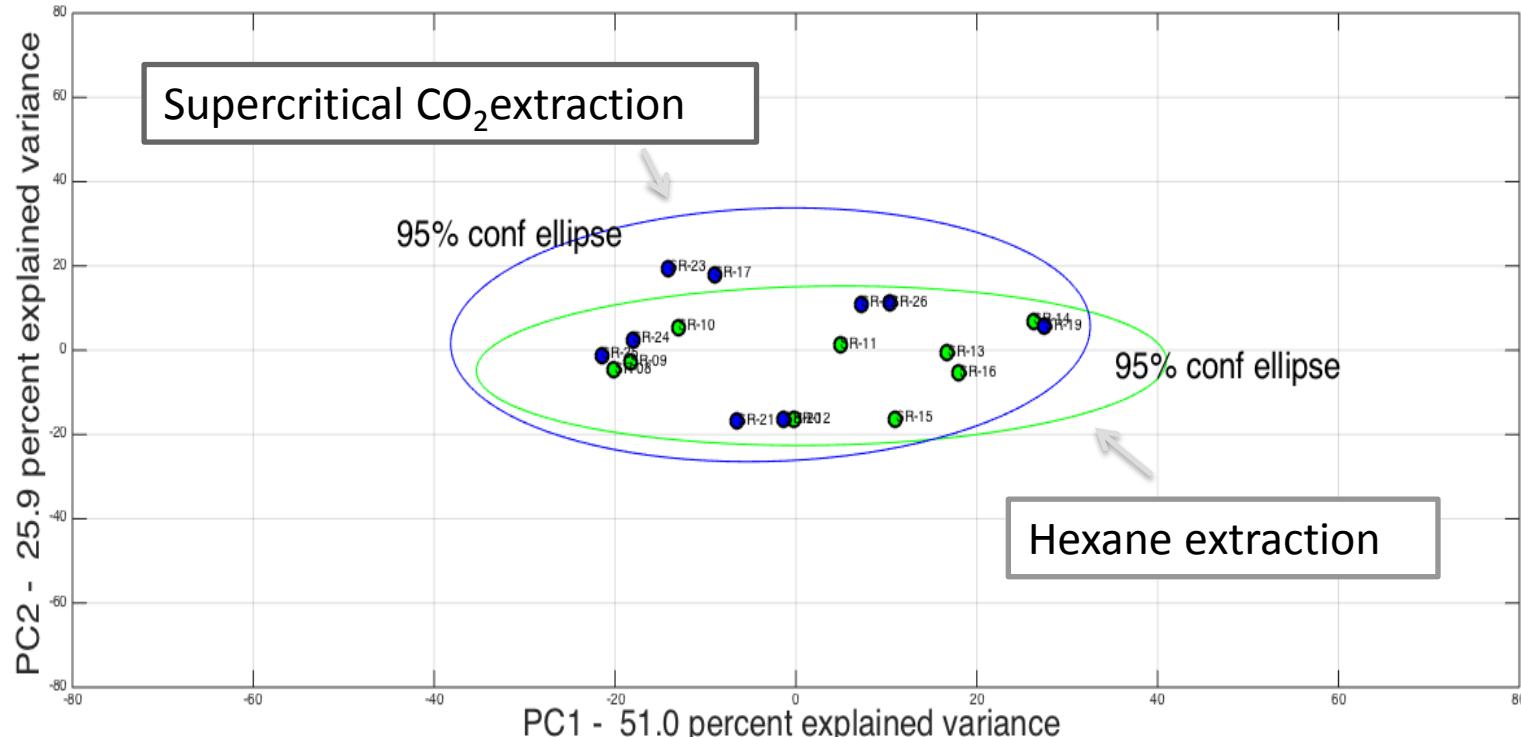


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## PRINCIPAL COMPONENT ANALYSIS (PCA)

*SERENOA REPENS* EXTRACT (CO<sub>2</sub> SUPERCRITICAL AND  
HEXANE EXTRACTS PHYTOEQUIVALENCE VERIFICATION)

### NMR FINGERPRINTING (QC) – PCA ANALYSIS



R. Pace et al.; *Fitoterapia*, 102(2015)56-60

## POTENTIAL USE OF PCA

Can be used to support genuinity of the extracts

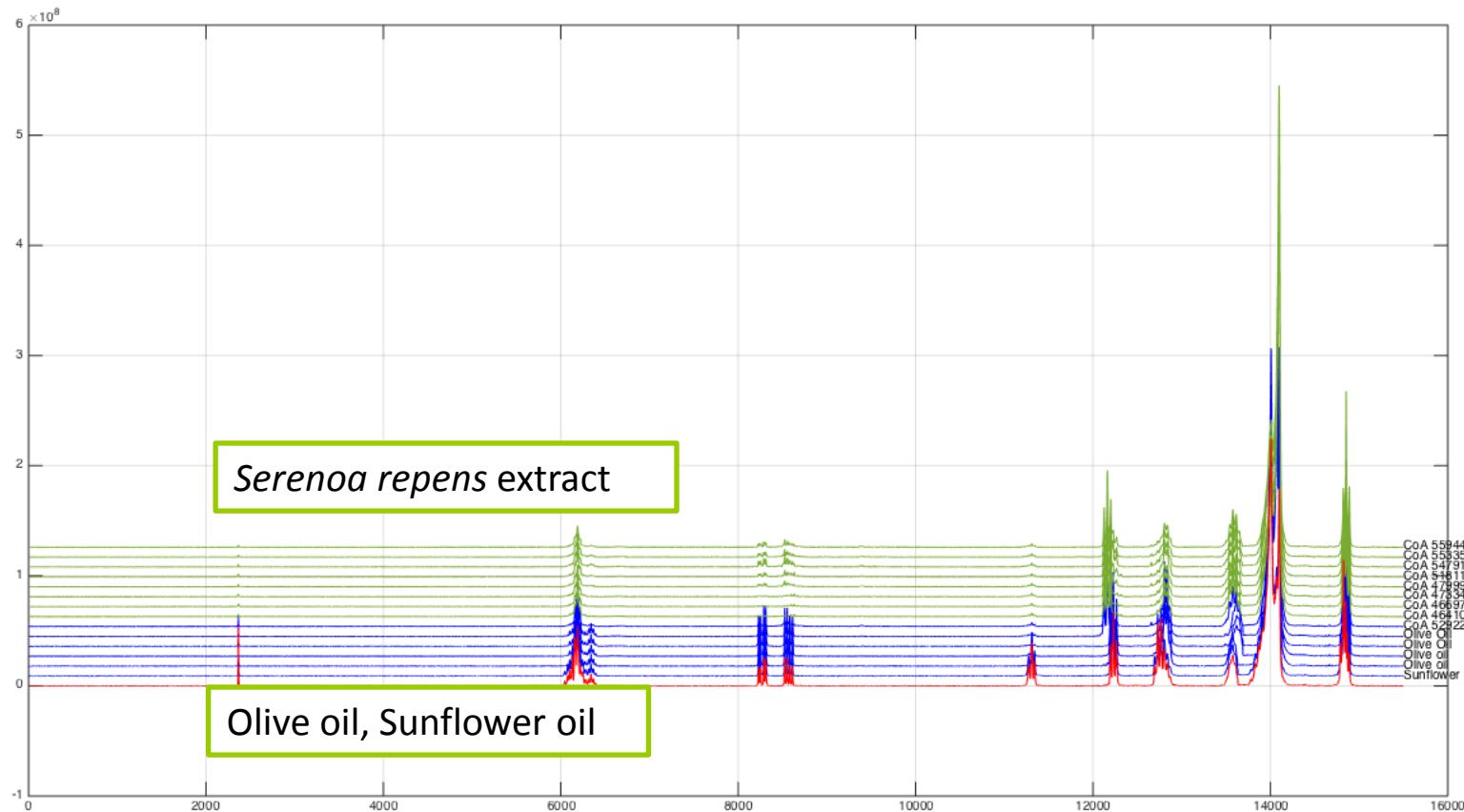
or

To put into evidence potential adulterations

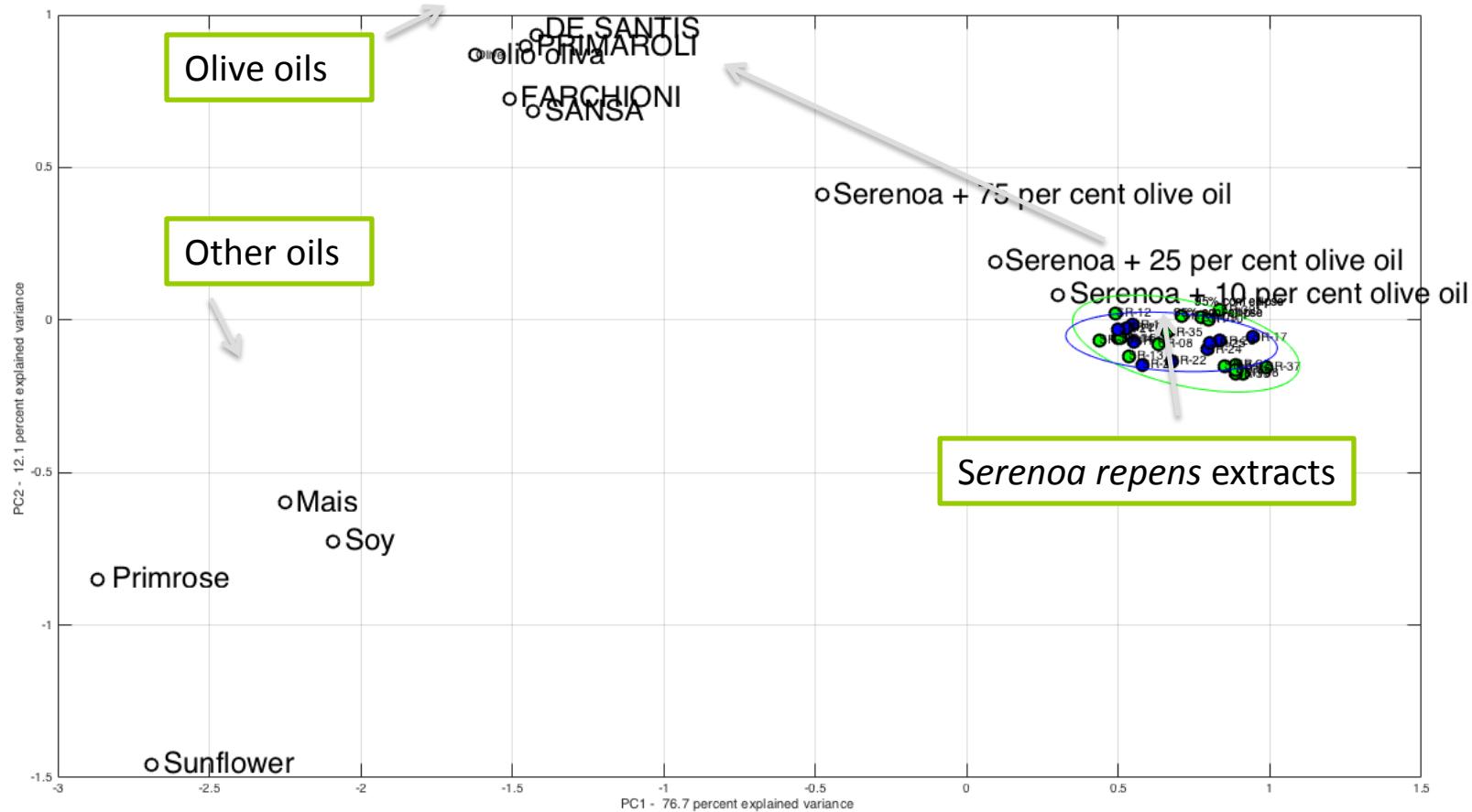
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## FINGERPRINTING VARIABILITY - PCA

SERENOA REPENS EXTRACT  
NMR FINGERPRINTING



# USE OF PCA FOR HERBAL EXTRACTS ADULTERATION DISCOVERY



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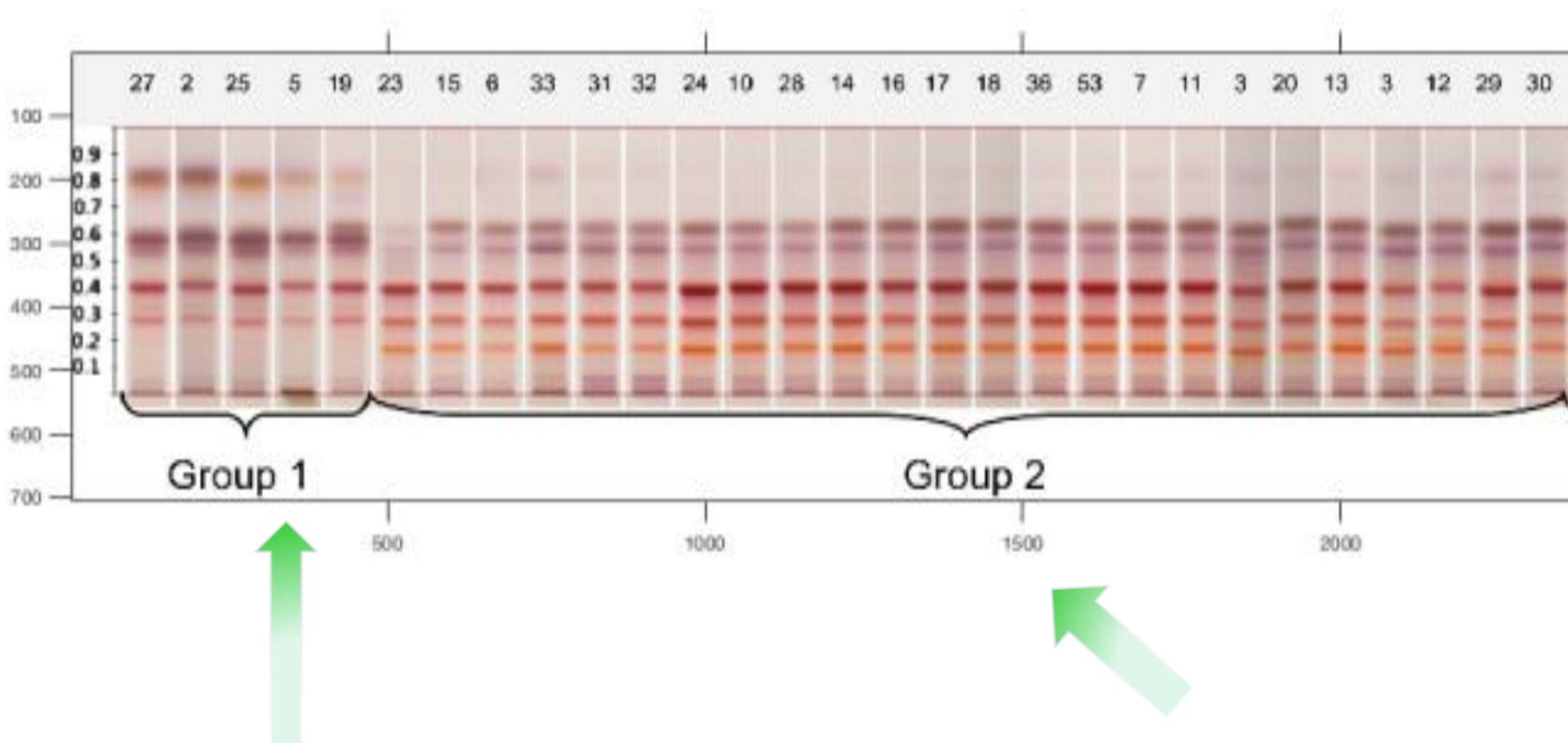
# PCA AS TOOL FOR OVERALL HERBAL EXTRACTS VARIABILITY EVALUATION

Can be used for statistical image comparison like  
HPTLC of plants or extracts

## HPTLC profile of *Curcuma* spp

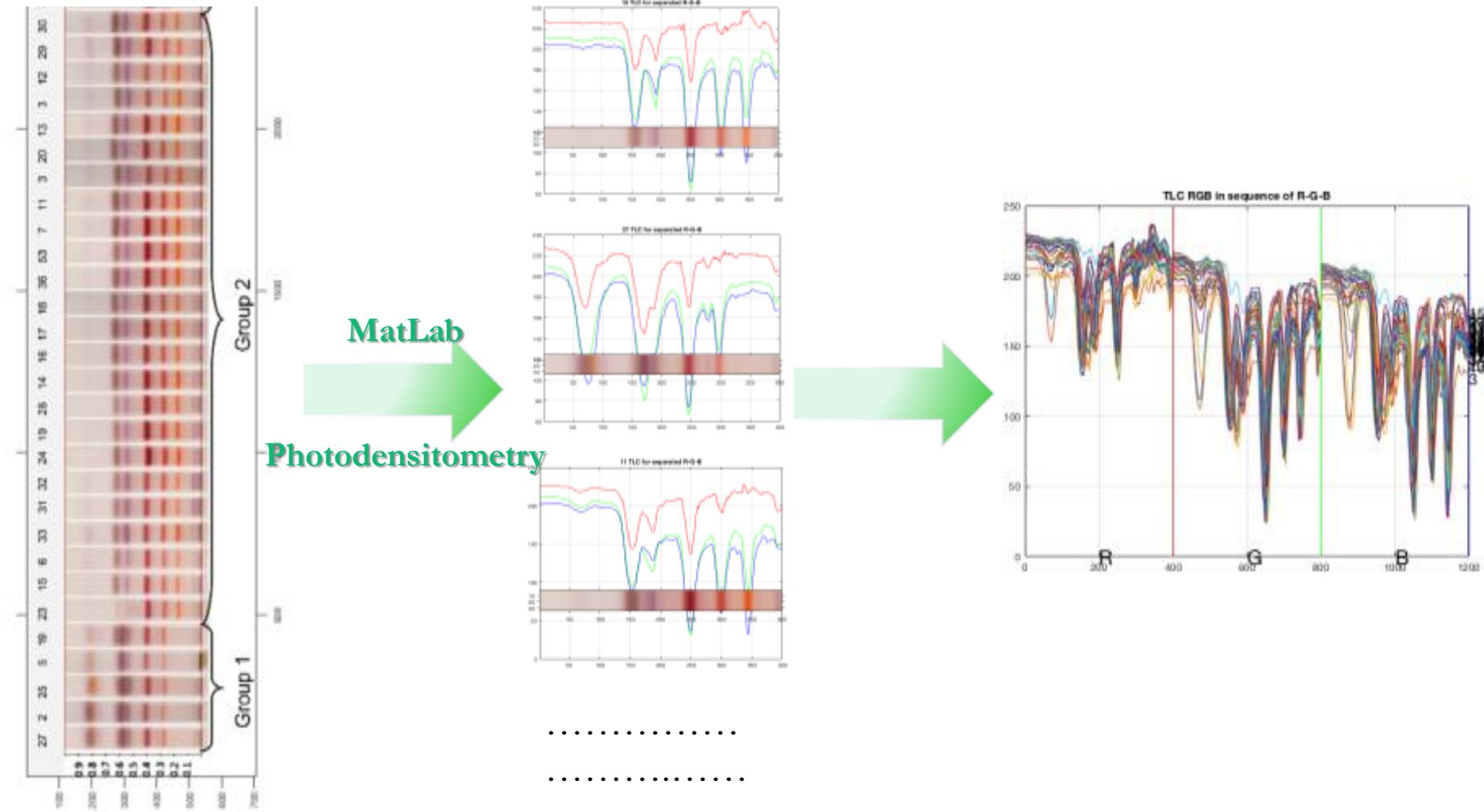
HPTLC is widely used in the Authentication and QC of herbals

Ref.: Anthony Booker et al., Journal of Ethnopharmacology, 152 (2014) 292-301



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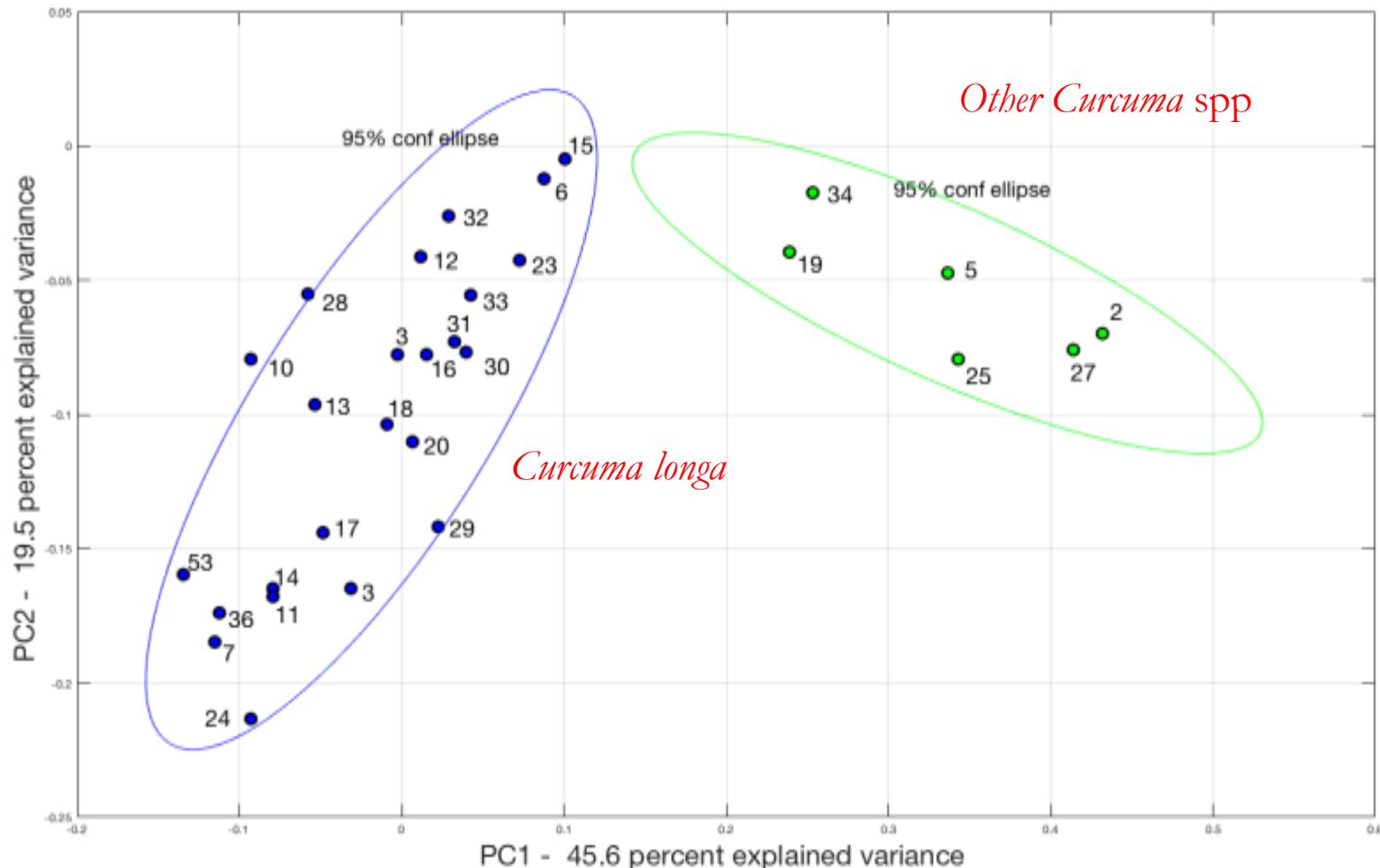
## HPTLC profile of Curcuma spp



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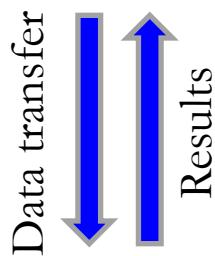
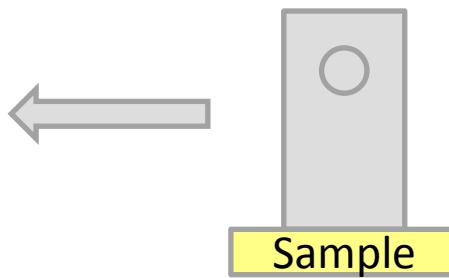
HPTLC profile of *Curcuma* spp

PCA

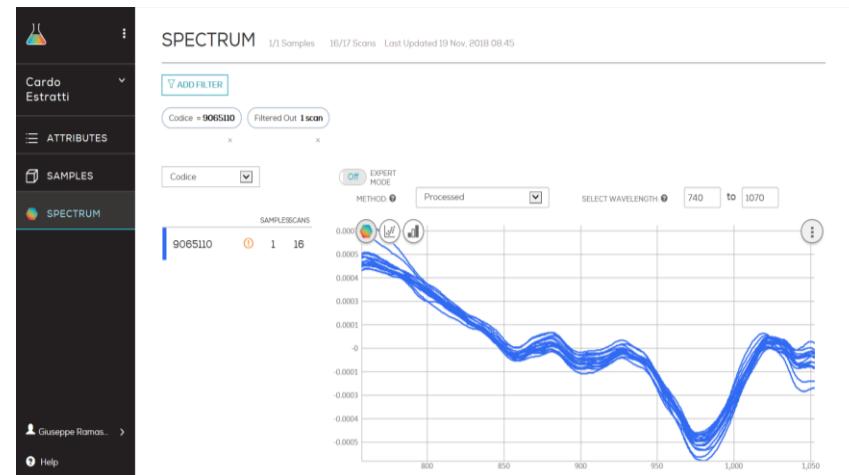


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Portable NIR  
700-1100 nm



Storage/Processing



# CONCLUSION

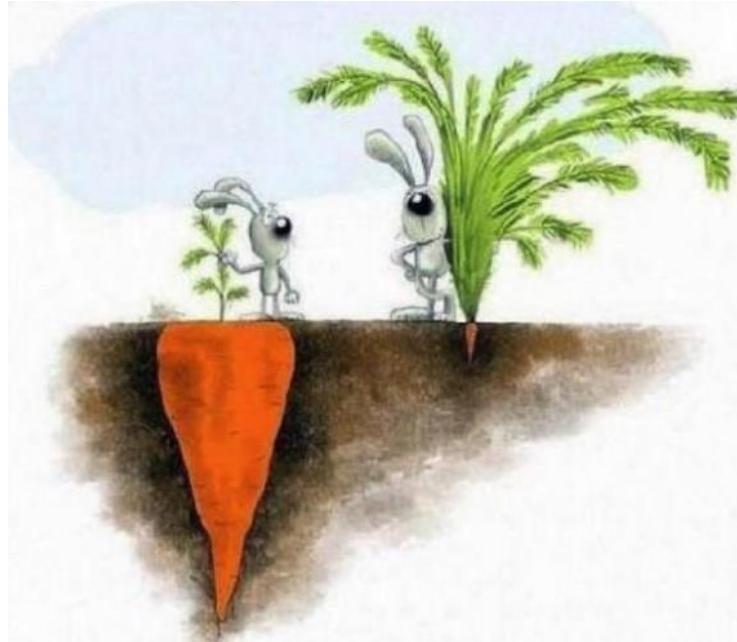
## REVISITING THE CONCEPT OF BOTANICAL THERAPEUTICS IN TERM OF QUALITY AND REGULATORY SUPPORT

**"When we stop improving,  
we start to slip backward." -**

H. James Harrington

# Modern Trends in Quality and Regulatory Aspects of Herbal Extracts

Quality...



... is not  
always what  
you see

