Simplification of Testing and Analytical Methods-Harmonization of Procedure

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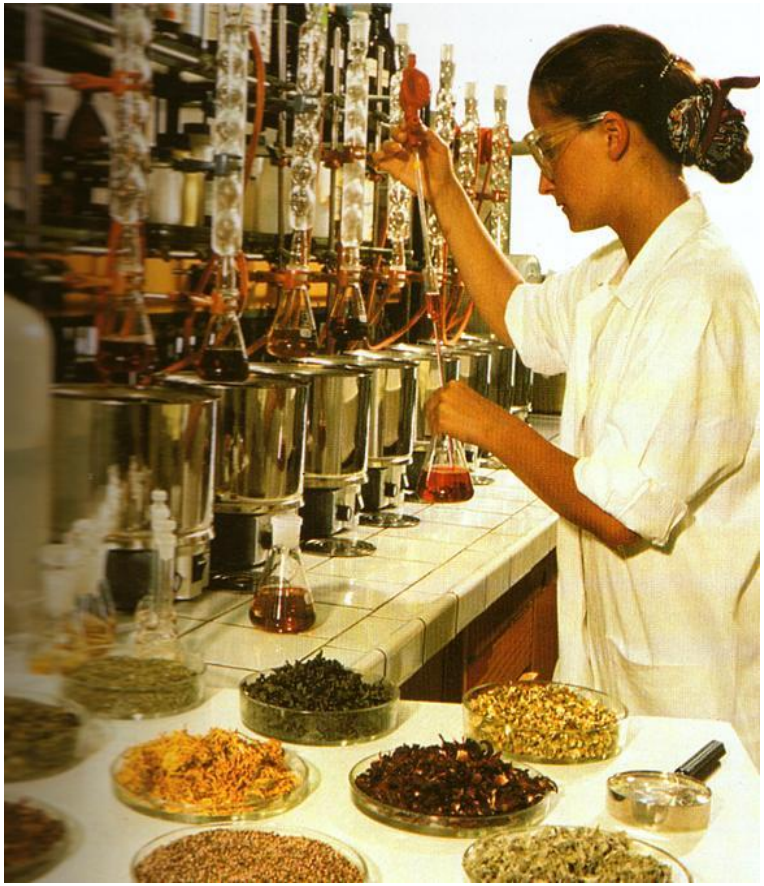
Spices & Herbs

- Spices and herbs have been used not only for flavoring foods but also for their antiseptic or medicinal properties since the prehistoric era.
- Flavoring substances such as aroma and taste are reviewed from the organoleptic aspects, followed by preservative effects of antimicrobial and antioxidant constituents.
- Bioactive components in spices and herbs may lead to chemoprevention of inflammation, cancer and other diseases in human organs as well as prevention of food deterioration.

Plant Parts and Spices & Herbs

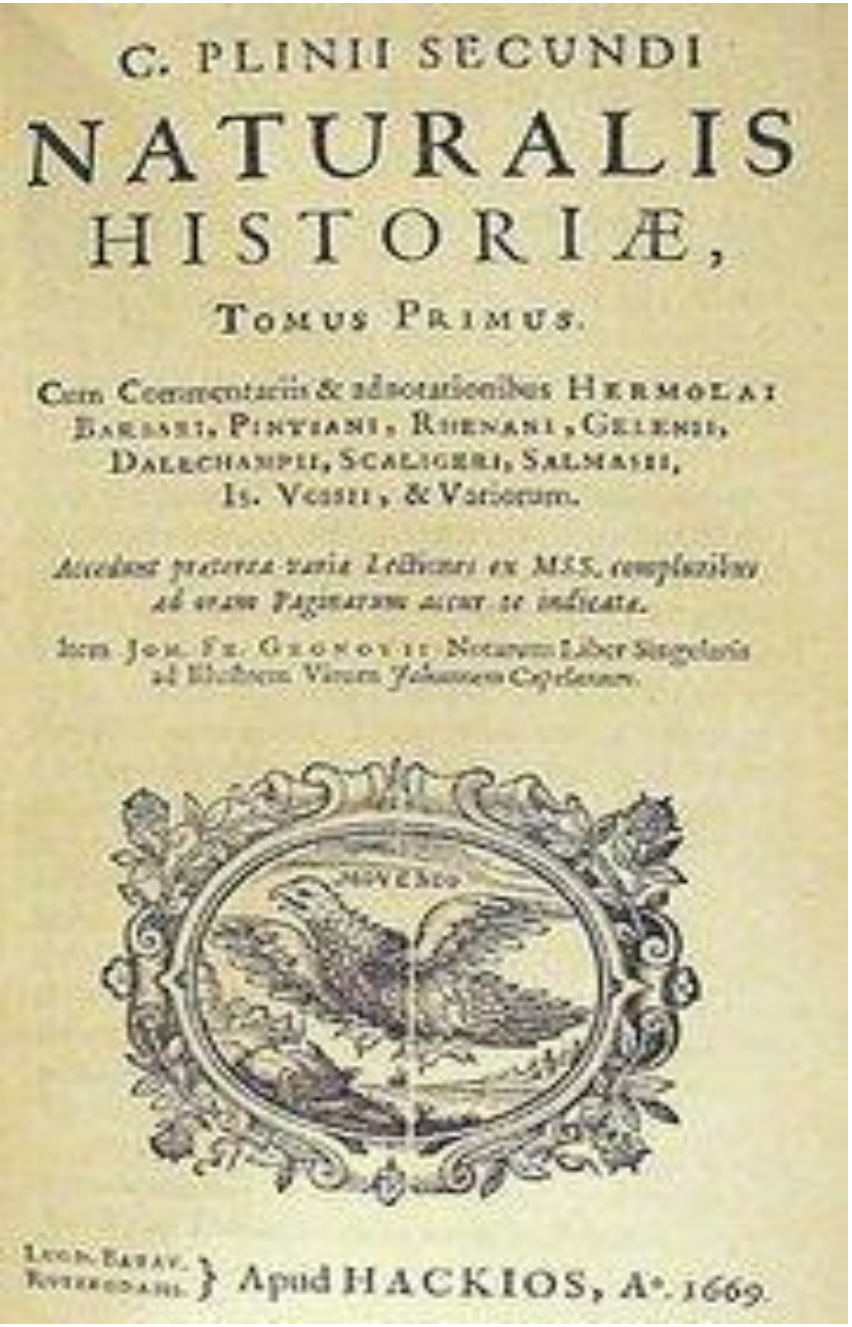
- **Barks**: Cassia and Cinnamon
- **Berries**: Black pepper and Pimento
- **Unopened flower buds**: Cloves
- **Bulbs**: Garlic and Onion
- **Floral parts**: Saffron
- **Leaves**: Bay leaves, Basil, Curry Leaves, Parsley, Sage, Thyme
- **Pods**: Vanilla, Tamarind
- **Seeds**: Aniseed, Cumin, Caraway, Celery, Fennel, Fenugreek, Mustard and Poppy Seeds
- **Resin or Exudate**: Asafoetida
- **Rhizomes**: Ginger and Turmeric

Quality Control



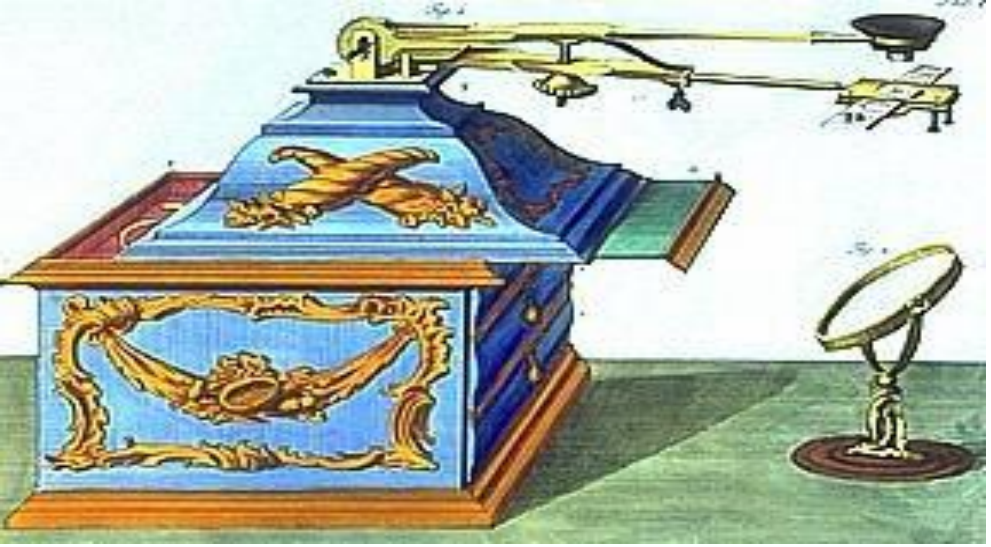
Herbs and Spices- Main Issues

- **Safety**
  - **Biological**
  - **Environmental**
  - **Chemical**
- **Quality (Authenticity?)**
  - **Standardization**
  - **Selection of marker compounds**
  - **Adulteration/misidentification**
- **Efficacy**


C. PLINII SECUNDI
NATURALIS
HISTORIÆ,
TOMUS PRIMUS.
Cum Commentariis & adnotationibus HERMOLAI BARBARI, PINTIANI, RHENANI, GELENII, DALECHAMPII, SCALIGERI, SALMASII, Is. VOSSII, & Variorum.
Accedunt praterea varia Lectiones ex MSS. compluribus ad oram Paginarum accurate indicata.
Item JOH. FR. GRONOVII Notarum Liber Singularis ad Illustrem Virum Johannem Cyprianum.
LUGD. BATAV. ROTERODAMI. } Apud HACKIOS, A°. 1669.

Pliny the Elder circa AD 23-79

Frequently **commented on Adulteration** and advocated the use of **Organoleptic** test to detect their quality



Arthur Hill Hassle (1850s)

An English physician was the first to make use of systematic and comprehensive application of "**Microscopy**" to food and drug quality assessment/detection of adulteration.

Choosing the best method of sample analysis

TLC

GC

HPLC

CE

SFC


GOAL
ASSURING QUALITY AND SAFETY
Implementation
Education/ dissemination
Quality Assessment
Assuring Safety
Acquiring resources
Acquiring knowledge
Understanding
Harmonization

Approaches

- **Good Agricultural Practices (GAP)**
- **Spices/Herbs and chemical constituents**
- **Approach of rapid characterization**
  - Collecting information and authentic samples
  - Chemical reactions
  - TLC/HPTLC
  - IR/UV
  - Modern techniques (GC/MS, LC/MS)

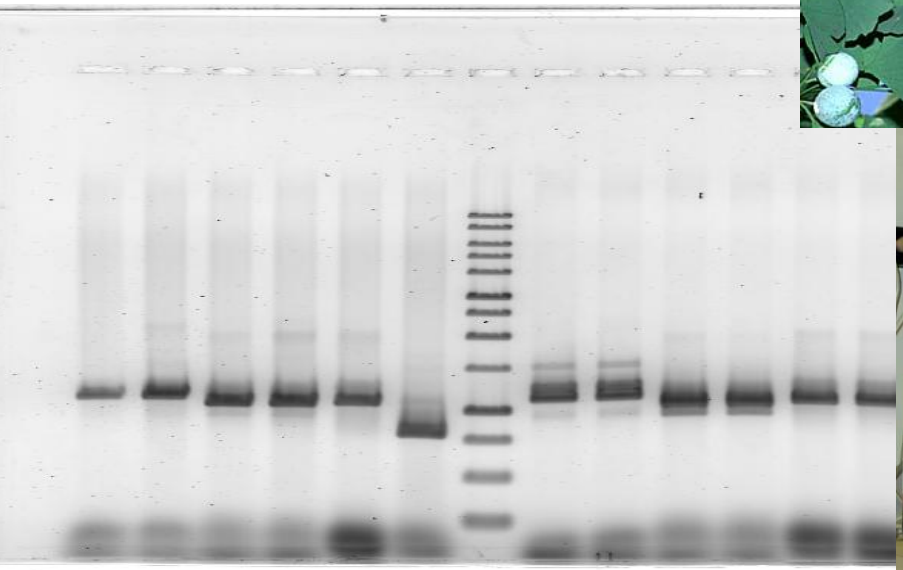
Approach for Rapid Characterization

MONOGRAPHS

- WHO monographs for medicinal plants
- ASTA (American Spice Trade Association)
- ISO (International Organization for Standardization)
- BSI (British Standards Institution)
- BIS (Bureau of Indian Standards)
- Others
  - Scientifically Valid Analytical Methods (Published Journal Articles)
  - Herbal Pharmacopeias

Authentication:

- **Organoleptic**
- **Macroscopic**
- **Microscopic**
- **Chemical Analysis**
- **Genetic Profiles**



Organoleptic

- **Organoleptic characters include comparison of Smell, Taste, Odor, Color with a "Reference standard"**

- Particularly useful in authentication of botanicals in powder form

Macro-Morphological Textures

Sea weed

Cacti

Root

Palm husk

Illicium-Who is who?

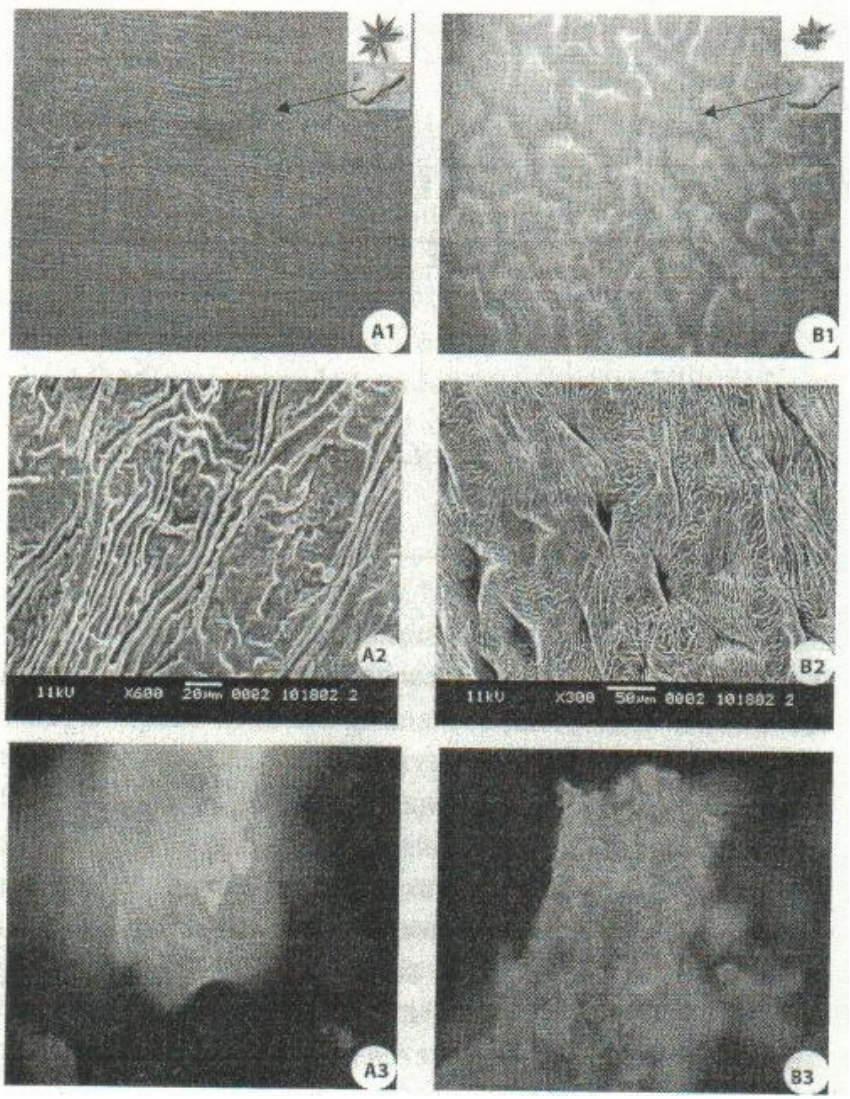
Illicium verum

Illicium anisatum

The characteristically regular eight-pointed shaped fruit capel contains one elipsoid, shiny, reddish brown seed with two cotyledones.

Picture from: http://www.finances.gouv.fr/DGCCRF/01_presentation/activites/labos/2001/images/badiane1.jpg

Star Anise (*Illicium verum*) vs. *Illicium spp.*



Microscopies of *I. verum* and *I. anisatum*

Surface view of follicular epicarp cells of *I. verum* (A1) and *I. anisatum* (B1) under fluorescent microscope; surface view of follicum epicarp cells of *I. verum* (A2) and *I. anisatum* (B2) under SEM; *I. verum* (A3) and *I. anisatum* (B3) as seen in powdered mixture (1:1) under fluorescent microscope

The University of Mississippi

Vaishali C. Joshi, Pullela.V. Srinivas and Ikhlas. A. Khan, *J. AOAC Int.*, 2005, 88(3), 703-706

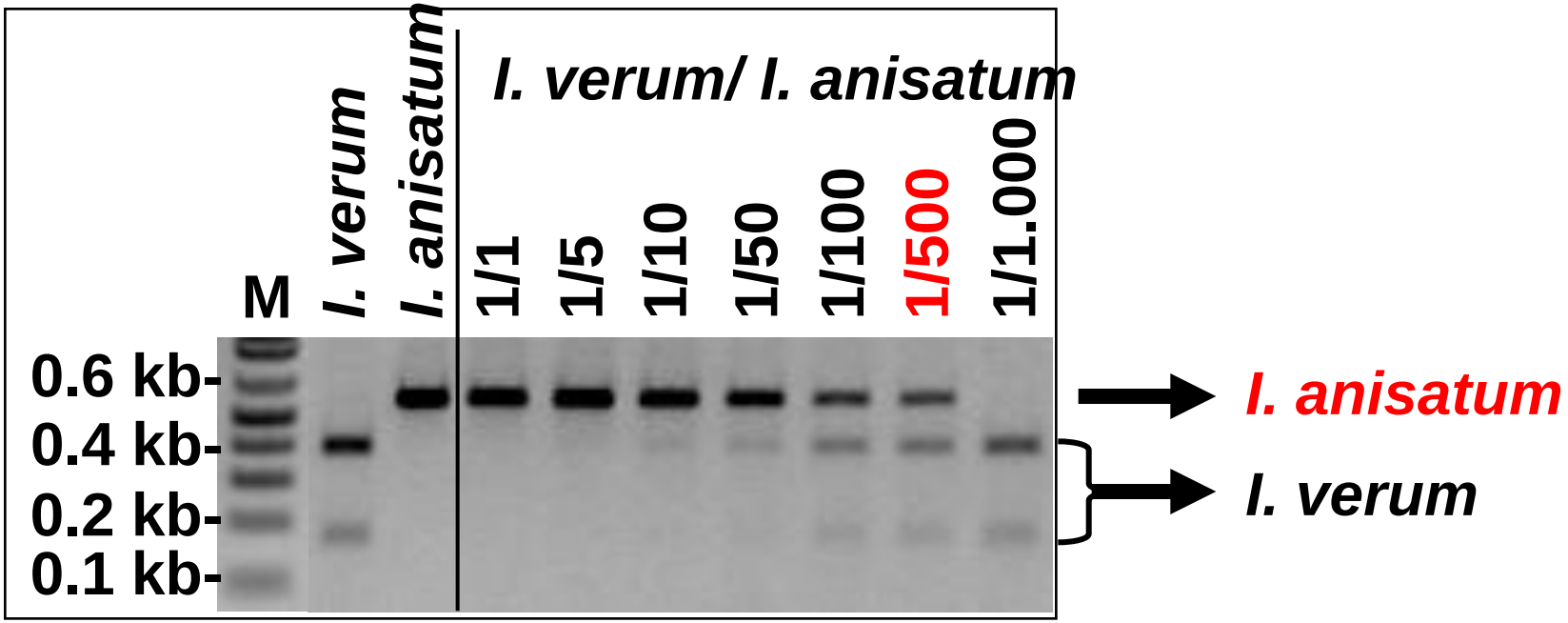
Adulterant present?

Ratios of I. verum/ I. anisatum tissue

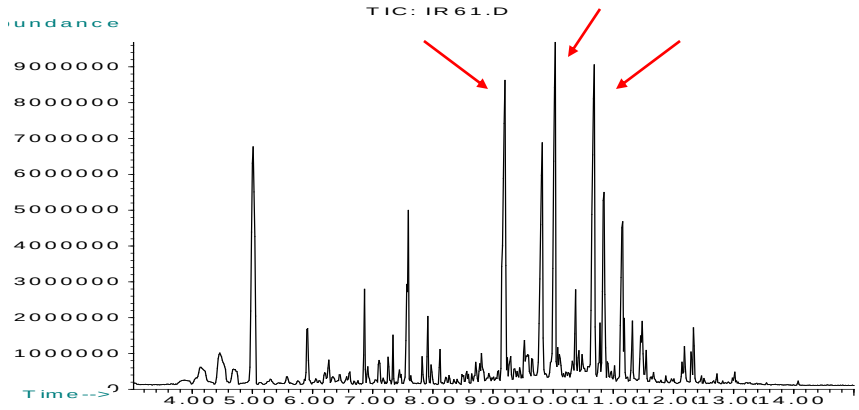
1/1 1/10 1/50 1/500 1/1.000

It is very difficult to detect *Illicium anisatum* fruit as adulterant of powdered *Illicium verum*. A molecular marker can help.

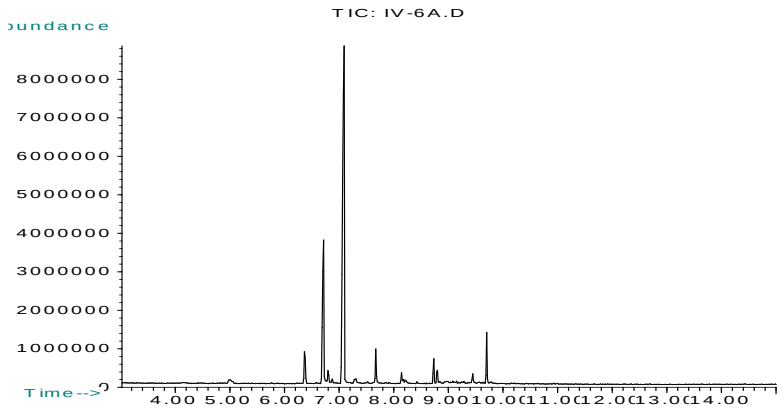
Detection of adulterant *I. anisatum in I. verum*



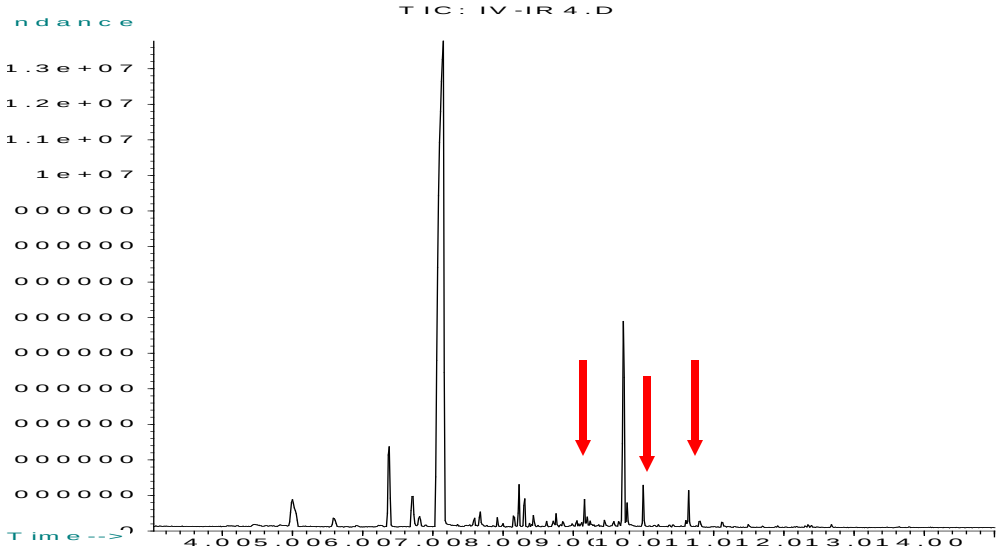
PCR product was digested with restriction endonuclease *Pst*I. Results indicate that as little as 1/500 part of *Illicium anisatum* tissue can be detected in *I. verum/I. anisatum* mixtures. Compared to *I. anisatum* low amount of *I.verum* product might be due to less copy numbers of ITS



I. anisatum



I. verum



***Powdered mixture of I. verum* and *I. anisatum* (9:1)**

TLC/HPTLC & Spices and Herbs

- High Performance of Thin Layer Chromatography (HPTLC) is an ideal analytical tool for herbal complex mixture analysis.
- TLC/HPTLC methods offer the option of presenting the results as an image.
- HPTLC can be used for qualitative as well as quantitative analysis
- Qualitative analysis
    - Fingerprint
    - Screening
    - Identification
- Quantitative analysis
- One-time or routine use

IR/UV & Spices and Herbs

- Infrared spectroscopy (IR) and Ultraviolet-visible spectroscopy (UV-vis) are be used as analytical tools in the analysis of herbal samples.
- IR/UV-vis methods can analyze samples that contain pure or very simple component.
- IR/UV-vis methods can be used for quantitative analysis

Modern Techniques & Spices and Herbs

- Modern techniques use chromatography to separate the analytes and identify them with different detectors.
- Commonly used modern techniques include gas chromatography (GC), high performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC).
- Detectors refer to photodiode array detector (PDA), evaporative light scattering detector (ELSD), fluorescence detector, mass spectrometer, and electro chemical detector.

Summary

- Analytes and information of spices and herbs need to be clarified.
- Sample preparation is necessary for rapid identification of spices and herbs.
- Chemical reactions are useful for rapid characterization of functional groups of analytes from spices and herbs.
- TLC/HPTLC is a rapid tool for herbal complex mixture analysis and characterization.

Collaboration/ Training

Introduction

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use.

Validation of analytical methods is an essential but time-consuming activity for most analytical development laboratories.

VALIDATION PROTOCOLS

- Introduction
- Why Validation
- Validation Characteristics
  - Accuracy
  - Precision
  - Specificity
  - Detection limits
  - Quantitation limits
  - Linearity
  - Range
  - Robustness
- Information Required in Analytical Procedur
- Method Verification
- Method Revalidation

References

WHY VALIDATE ANALYTICAL PROCEDURES

There are many reasons for the need to validate analytical procedures.

[1] regulatory requirements,

[2] minimize analytical & instrumental errors,

[3] quality control requirements

[4] reliable & reproducible results

[5] to be assured of the correctness of results

Verification Tools:

- **Blanks:** various types of blanks usage
- **Reference materials and certified reference materials**
- **Fortified (Spiked) Materials and Solutions:** Recovery determinations
- **Standards:** used for calibration and determination purpose
- **Replication:** Replicate analysis to check for changes in precision which may affect results
- **Statistics:** to evaluate accuracy, precision, linear range, LOD and LOQ, measurement uncertainty

Sampling:

- Analysis starts with sampling
- For trace analysis: sampling becomes a major source of error
- Differs from matrix to matrix

Sampling

Sample preparation

Analysis

Calibration

Analyzing qualitative data

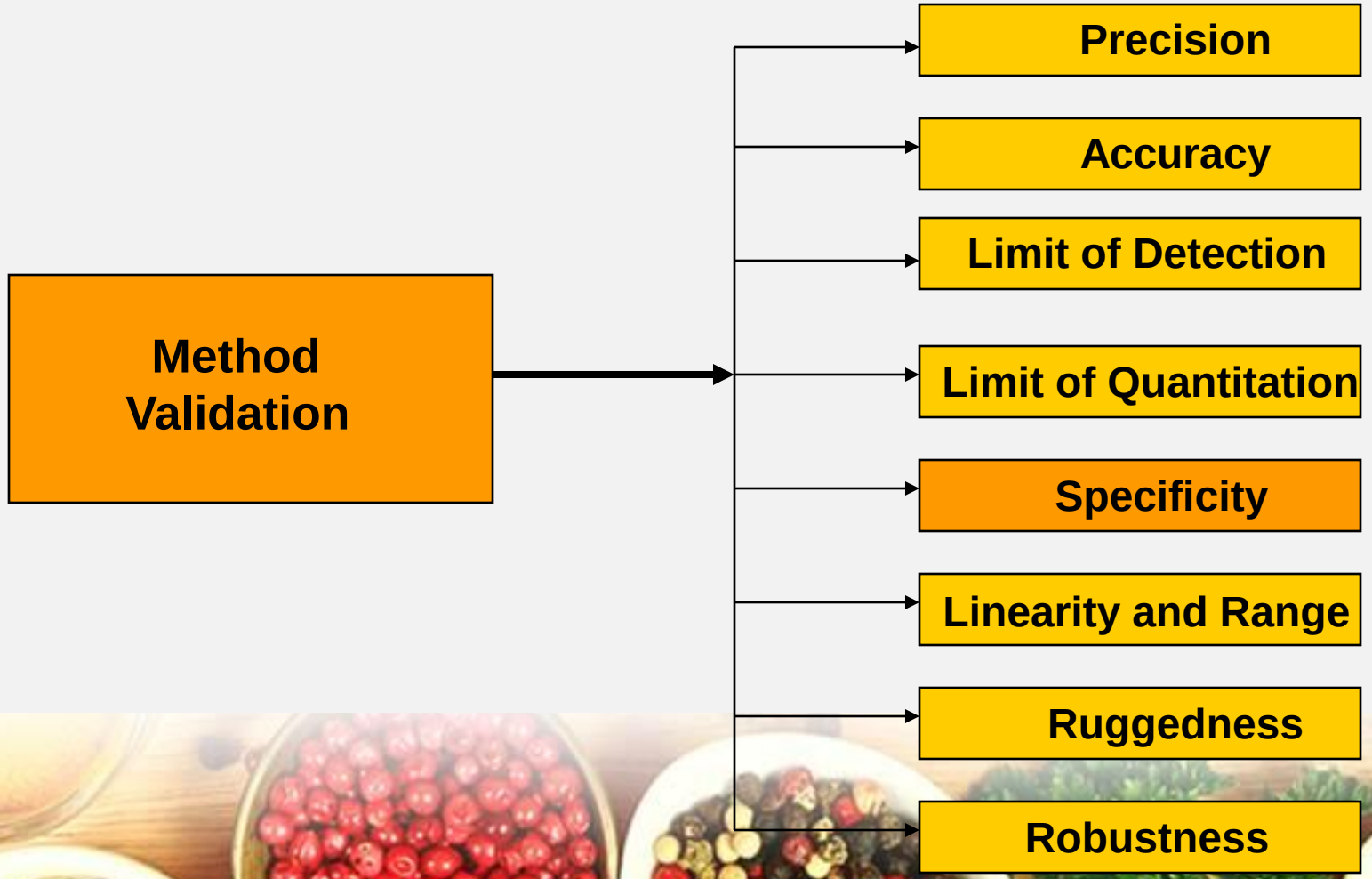
How do I summarize and make sense of all these words?

Data Evaluation & Interpretation

Reporting

Method Validation

There are 8 steps of method validation according to the specifications listed in USP:



SINGLE LAB VALIDATION

http://www.aoac.org/Official_Methods/slv_guidelines.pdf
http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm0887

MULTI-LAB VALIDATION

http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm0887

Method Verification

- importing a validated method
- show that laboratory can do it at its site
- demonstrate that laboratory can repeat the method performance

METHOD REVALIDATION

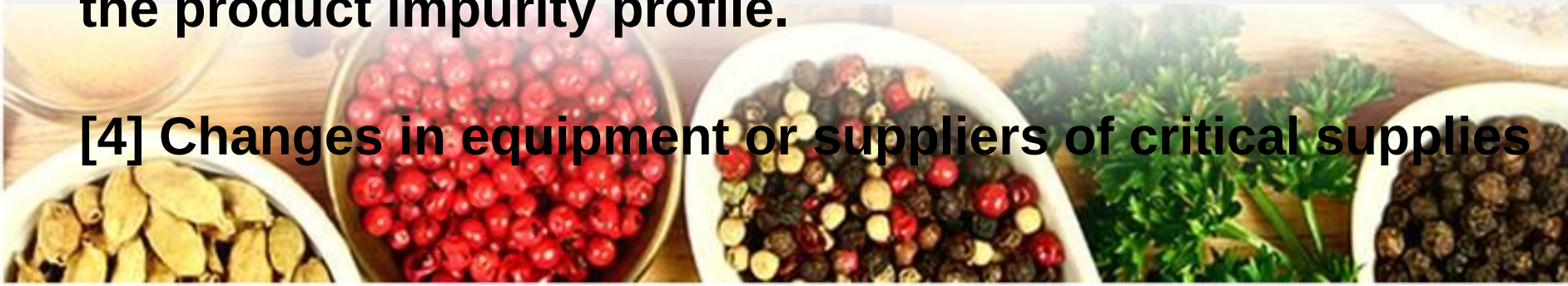
Common situations where the method has to be revalidated are:

[1] Significant changes in the process.

[2] If a new impurity is found that makes the method deficient in its specificity, this method will need to be modified or redeveloped and revalidated to ensure that it will be able to perform its intended function

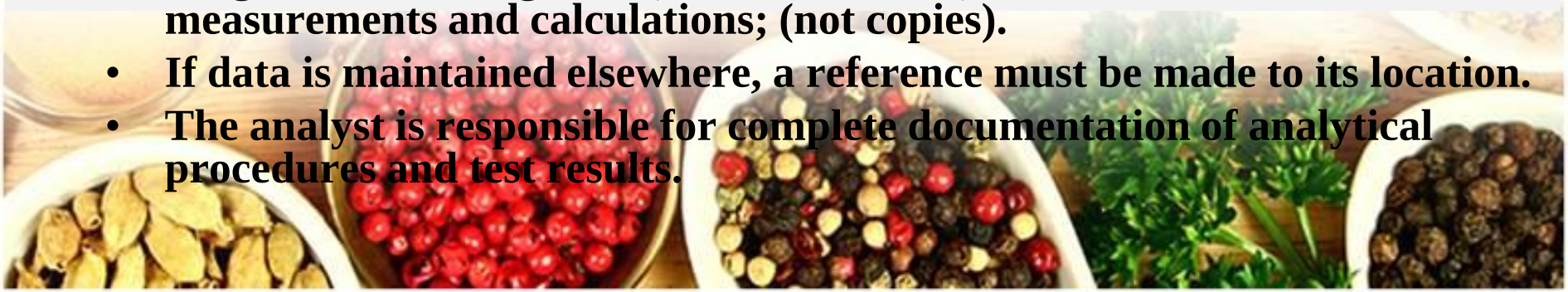
[3] A change in the excipient composition may change the product impurity profile.

[4] Changes in equipment or suppliers of critical supplies



Documentation–GLP

- If it isn't written down, It didn't happen!
- Full description of standard, lot #, purity, exp. date
- Full description of sample, dose, type of formulation, labeling and packaging etc.
- Full identification of chemicals, solvents, TLC plates with lot #, equipment, etc.
- Full description of reagent and/or mobile phase preparation.
- Full description of sample and standard preparation.
- Formulas used for calculations and sample calculations.
- Describe experimental observations and include conclusions and/or results of the test.
- Use a notebook or worksheet for recording all raw data.
- Strikeouts must be initialed, dated and reason given for the strikeout.
- Test method must be referenced.
- Original chromatograms (TLC, HPLC etc) must be used for measurements and calculations; (not copies).
- If data is maintained elsewhere, a reference must be made to its location.
- The analyst is responsible for complete documentation of analytical procedures and test results.



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Prev.

Cassia Cinnamon as a Source of Coumarin in Cinnamon-Flavored Food and Food Supplements in the United States

Yan-Hong Wang †, Bharathi Avula †, N. P. Dhammika Nanayakkara †, Jianping Zhao †, and Ikhlas A. Khan *†‡§

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Publication Date (Web): April 12, 2013

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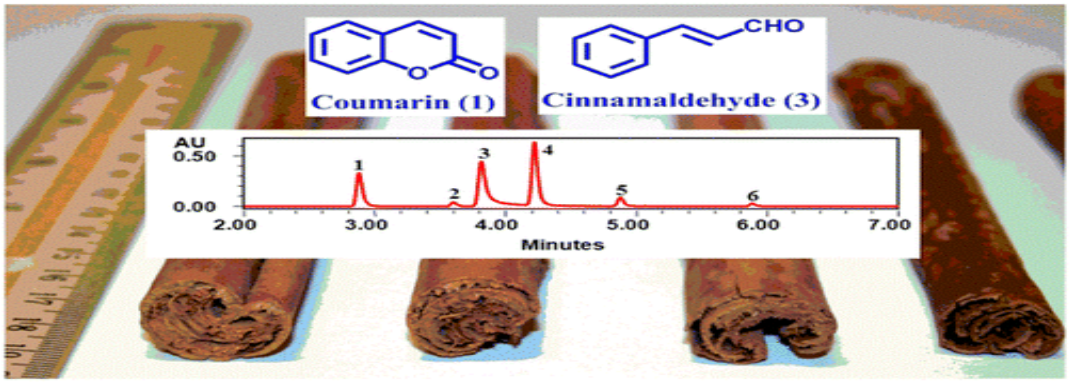
Section: Food and Feed Chemistry

Abstract



Coumarin as an additive or as a constituent of tonka beans or tonka extracts is banned from food in the United States due to its potentially adverse side effects. However, coumarin in food from other natural ingredients is not regulated. "True Cinnamon" refers to the dried inner bark of *Cinnamomum verum*. Other cinnamon species, *C. cassia*, *C. loureiroi*, and *C. burmannii*, commonly known as cassia, are also sold in the U.S. as cinnamon. In the present

The Wall Street Journal Oct 14, 2013

Little Bit of Spice for Health, but Which One?

While Ceylon Cinnamon Is Milder Than Grocery-Store Variety, There Are Few Studies on Its Benefits

By
LAURA JOHANNES

What's the difference between Ceylon and cassia cinnamon?

Which is better? How do you tell them apart? (And what's all the fuss about?)

By Melissa Breyer

Tue, Jan 21, 2014 at 5:39 PM

Related Topics: Diabetes, Health & Well Being, Healthy Eating, Alternative Medicine

Photo: sutsaly/Shutterstock

Cinnamon — the charming spice that gives Red Hots their kick and cinnamon buns their name — has reached superfood status, owing to its numerous health benefits. Scientists told us that regular consumption of cinnamon could potentially lower blood sugar, help digestion, ease arthritis, lower cholesterol and even ward off Alzheimer's.

WORLD NOW

EU to Denmark: Drop that cinnamon roll! Or at least the spice level

Danish cinnamon rolls, like the ones displayed here at a Copenhagen bakery, contain too much cinnamon, according to the European Union's food safety experts. The cassia type of spice favored in Scandinavian baking contains trace amounts of a chemical compound called coumarin that has been linked to liver damage in a small number of sensitive people. (Folkver Per / Associated Press / August 4, 2010)

Wednesday January 29, 2014

chron

Cinnamon may interact with medications

By Joe Graedon, M.S., and Teresa Graedon, Ph.D. | January 17, 2014 | Updated: January 17, 2014 6:52pm


Laurus nobilis
Umbellularia californica
Cinnamomum tamala
Pimenta racemosa
Syzygium polyanthum
Murraya koenigii
What kind of Bay Leaf are we talking about?!
Cinnamomum tamala
Pimenta racemosa
Syzygium polyanthum
Umbellularia californica
Syzygium polyanthum
Laurus nobilis
Pimenta racemosa

Thank You!

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