Simplification of Testing and Analytical Methods - Harmonization of Procedure

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• 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
Pliny the Elder  circa AD 23-79

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

HPLC

CE

GC

SFC
HARMONIZATION

ASSURING QUALITY AND SAFETY

Implementation
Education/ dissemination
Quality Assessment
Assuring Safety

Acquiring resources
Acquiring knowledge
Understanding
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.

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

Adulterant present?

Ratios of *I. verum* / *I. anisatum* tissue

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Image</th>
</tr>
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<tbody>
<tr>
<td>1/1</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>1/10</td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>1/50</td>
<td><img src="image3.png" alt="Image" /></td>
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<td>1/500</td>
<td><img src="image4.png" alt="Image" /></td>
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<tr>
<td>1/1,000</td>
<td><img src="image5.png" alt="Image" /></td>
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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 *PstI*. 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 regions in this species.
*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 used as analytical tools in the analysis of herbal samples.
- IR/UV-vis methods can analyze samples that contain pure or very simple components.
- 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 electrochemical 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 Procedure
• 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 → Data Evaluation & Interpretation → Reporting

Analyzing qualitative data

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

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

- Precision
- Accuracy
- Limit of Detection
- Limit of Quantitation
- Specificity
- Linearity and Range
- Ruggedness
- Robustness
SINGLE LAB VALIDATION

MULTI-LAB VALIDATION
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.

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

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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
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: sutsaiv/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.
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. (Polfoto Per / Associated Press / August 4, 2010)
Cinnamon may interact with medications

By Joe Graedon, M.S., and Teresa Graedon, Ph.D. | January 17, 2014 | Updated: January 17, 2014 6:52pm
What kind of **Bay Leaf** are we talking about?!
Thank You!