Characterization of Botanical Extracts Using Multiple Technologies

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The three main components of the Botanical Integrity (BI) model are: botanical examination (botany), phytochemical analysis (chemistry), and biological and safety assessment (bioactivity). The concerted use of multiple methodologies from all three components is required to obtain a comprehensive representation of a botanical material.

National Center for Complementary and Integrative Health (NCCIH, formerly NCCAM) and the Office of Dietary Supplements (ODS), both at the US National Institutes of Health (NIH).
## TLC vs. HPTLC

Comparison between TLC (left) and HPTLC (right) analyses of Melissa officinalis extracts

**Mobile phase:** n-hexane, ethyl acetate (9:1).

**Derivatization:** Anisaldehyde.

**Visualization:** UV 366.

**Tracks:** 1-3 marketed hydroalcoholic extracts, 4 mixture of essential oil constituents (citral, limonene, myrcene).

<table>
<thead>
<tr>
<th></th>
<th>TLC</th>
<th>HPTLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average particle size</td>
<td>10-15 µm</td>
<td>5-7 µm</td>
</tr>
<tr>
<td>Particle size distribution</td>
<td>wide</td>
<td>narrow</td>
</tr>
<tr>
<td>Separation distance</td>
<td>100 - 150 mm</td>
<td>30 - 70 mm</td>
</tr>
<tr>
<td>Running time</td>
<td>30 -200 min</td>
<td>3 - 20 min</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>50 ml</td>
<td>5 - 10 ml</td>
</tr>
<tr>
<td>Detection limit, absorb.</td>
<td>100 - 1000 ng</td>
<td>10 - 100 ng</td>
</tr>
<tr>
<td>Detection limit, fluoresc.</td>
<td>1 - 100 ng</td>
<td>0.1 - 10 ng</td>
</tr>
</tbody>
</table>
HPTLC plate photo (A) and 3D overlay of the chromatograms (B) of Carissa carandas ripe fruits collected from different geographical regions with ursolic acid at 366 nm.

**Track details:** 1: Ratnagiri, 2: **Rajapur**, 3: Dapoli, 4: **Lonavla**, 5: Ursolic acid (10 µg/mL), 6: Karjat, 7: Malshej, 8: Kalyan, 9: Igatpuri

Detection of Adulteration

Detection of adulteration of products of *Curcuma longa* rhizome with the anti-inflammatory drug nimesulide

**Derivatization reagent:**

2,5-Dichloro-1,4-benzoquinone reagent

**Track 1:** *Curcuma longa* rhizome (not spiked)

**Track 2:** *Curcuma longa* rhizome (spiked with 1% nimesulide)

**Track 3:** *Curcuma longa* rhizome (spiked with 2% nimesulide)

**Track 4:** *Curcuma longa* rhizome (spiked with 5% nimesulide)

**Track 5:** *Curcuma longa* rhizome (spiked with 10% nimesulide)
Quality Evaluation of Herbal Products

HPTLC fingerprints of extracts of similar marketed species of feverfews.

Derivatization: Anisaldehyde.
Track Details: 1-5: marketed products with feverfew reported in the label, 6: feverfew extract used as reference, 7: the same extract of track 6 after elimination of chlorophylls and other lipids, 8: Mexican feverfew extract used as reference, 9: the same extract of track 8 after elimination of chlorophylls and other lipids, 10: rutin

TLC’s Renaissance

TLC-LESA (Liquid Extraction Surface Analysis)-MS has a high potential for medium-polar compounds separated on reversed-phase TLC plates, but limitations are present when very apolar compounds have to be extracted.


Can be connected to any LC-MS system. Most types of TLC layers can be used. Extraction into vials is also possible.

Direct Analysis in Real Time (DART)
TLC-MS: A Powerful Combination

Analysis of triterpenoids and phytosterols in vegetables by thin-layer chromatography coupled to tandem mass spectrometry

HPTLC Identification of Ashwagandha (root)

Ashwagandha (root) (*Withania somnifera*)

**Track 1:** *Withania somnifera* extract, **Track 2:** *Withania somnifera* root, **Track 3:** *Withania somnifera* root, **Track 4:** *Withania somnifera* root, **Track 5:** β-Sitosterol, **Track 6:** Withanoside IV, **Track 7:** Withanolide A, **Track 8:** Withanone, **Track 9:** Withanolide D, **Track 10:** Withaferin A

AHPA Botanical Identity References Compendium
HPLC Profile of Ashwagandha (root)

Ashwagandha (root) (*Withania somnifera*)

**Column:** 25-cm x 4.6-mm, 5 um, PhenomenexLuna C18
**Detection:** UV, 227 nm

AHPA Botanical Identity References Compendium
GC Analysis of Lemon Essential Oil

Column: SLB-IL59, 30 m x 0.25 mm I.D., 0.2 µm
Detector: FID
Root of *Euphorbia fischeriana*, has been used for the treatment of edema, phlegm accumulation, inflammation, ascites and cancer in clinical practice for many years and has shown great efficacy.

Based on the previous studies, this plant mainly contains diterpenoids, triterpenoids and steroids.

Terpenoids, which have an isoprene or isopentane type skeleton, are considered the major constituents and the main bioactive ingredients.

The herb (*Euphorbia fischeriana*) and chemical structures of its five main bioactive ingredients: (A) Scopoletin; (B) 2,4-Dihydroxy-6-methoxy-3-methylacetophenone; (C) 17-Hydroxyjolkinolide B; (D) Jolkinolide B; and (E) Jolkinolide A.

Wenjing Li et al., *Molecules* 2017, 22, 1524
Representative ultra-performance liquid chromatography coupled with the quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS) chromatograms of: (A) Total ion chromatogram (TIC) of reference stock solution (8, Scopoletin; 18, 2,4-Dihydroxy-6-methoxy-3-methylacetophenone; 23, 17-Hydroxyjolkinolide B; 24, Jolkinolide B; and 29, Jolkinolide A); and (B) Total ion chromatogram (TIC) of extract sample obtained from Euphorbia fischeriana in positive-ion mode.

Wenjing Li et al., *Molecules* 2017, 22, 1524
Euphorbia fischeriana - Characterization

(13) Fischeroside A: $R_1=H$, $R_2=H$
(14) Fischeroside B: $R_1=H$, $R_2=\text{galloyl}$
(5) Fischeroside C: $R_1=\text{OH}$, $R_2=H$

(7) Ent-atisane-3β,16α,17-triol

(9) Kaurenoic acid
(11) β-Sitosterol
(20) Ebracteolatanolide A

Wenjing Li et al., *Molecules* 2017, 22, 1524
One or two markers or pharmacologically active components are commonly employed for evaluating the quality and authenticity of an herbal medicine.

Chromatograms of 17 extracts of *Ginkgo biloba* meet with the standard measured by UV Spectroscopy at wavelength of 318nm with satisfactory absorbance.

The score plot obtained by principal components analysis where PC1 means the scores coordinates of principal component 1 and PC2 the ones of principal component 2.

Liang et al., *Journal of Chromatography B* 2004, 812, 1–2, 53-70
The peak in the fingerprints of samples 2 and 3 around the retention time of 10 min is much higher than the one in the standard extract 17 and sample 8. This peak is rutin. In fact, rutin was added in the three outlier samples (1–3) in order to meet the old standard based on absorbance.

Liang et al., *Journal of Chromatography B* 2004, 812, 1–2, 53-70
Having taken care of the place of collection of the herb at proper time, having right smell, color and chemical composition, not infested by microorganisms, properly purified, potentiated and administered at proper dose and time, that medicine alone is considered as the ‘Best Medicine’.