Botanical Ingredient Adulteration

–

How Some Suppliers Attempt to Fool Commonly Used Analytical Techniques

Stefan Gafner, PhD

American Botanical Council

September 1, 2019

Innsbruck, Austria

350 universität innsbruck

Society for Medicinal Plant and Natural Product Research

Gesellschaft für Arzneipflanzen- und Naturstoff-Forschung e.V.

G A

Is Adulteration a Problem in Europe?

EU supplement law 'among world's strictest,' as study finds two thirds adulterated

By Will Chu
14-Aug-2019 - Last updated on 13-Aug-2019 at 14:49 GMT

An industry group declares the EU legal framework for food supplements 'among the strictest in the world,' in response to a study that finds almost two-thirds of supplements contain pharmacological active substances or plant toxins.

Taking Advantage of Unclear Taxonomy – *Euphrasia officinalis* Identification Challenges

- Species hybridize frequently
- Self-pollination and insect pollination → species may exhibit highly variable morphology (e.g., *E. minima*)
- Morphological distinction criteria often blurred (e.g., *E. rostkoviana* vs. *E. montana* or *E. versicolor*)
- Botanists use different scientific names for the same species

Euphrasia rostkoviana
(syn. *E. officinalis* subsp. *rostkoviana*)

Botanical Adulterants Program

American Botanical Council
Now in our 30th yr

The Peculiar Case of *Euphrasia odontites*

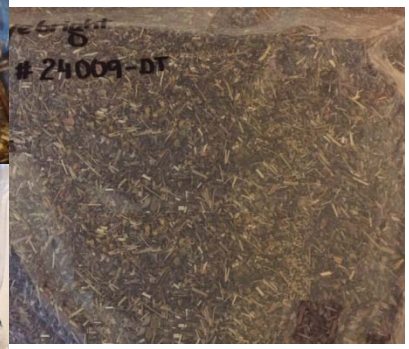
- Included in the Linnaean herbarium as *Euphrasia odontites*
- Species named *Euphrasia serotina* by Jean-Baptiste Lamarck in 1778 (illegimitate)
- Reclassified as *Odontites vulgaris* by Conrad Moench in 1794
- Reclassified as *Odontites serotinus* by Barthélemy Charles Joseph Dumortier (1827)
- Reclassified as *Odontites vernus* subsp. *serotinus* in 1893 by François Marie Louis Corbière

Common name: red bartsia
Still sold as *Euphrasia odontites* *or eyebright* today

Odontites vernus* subsp. *serotinus

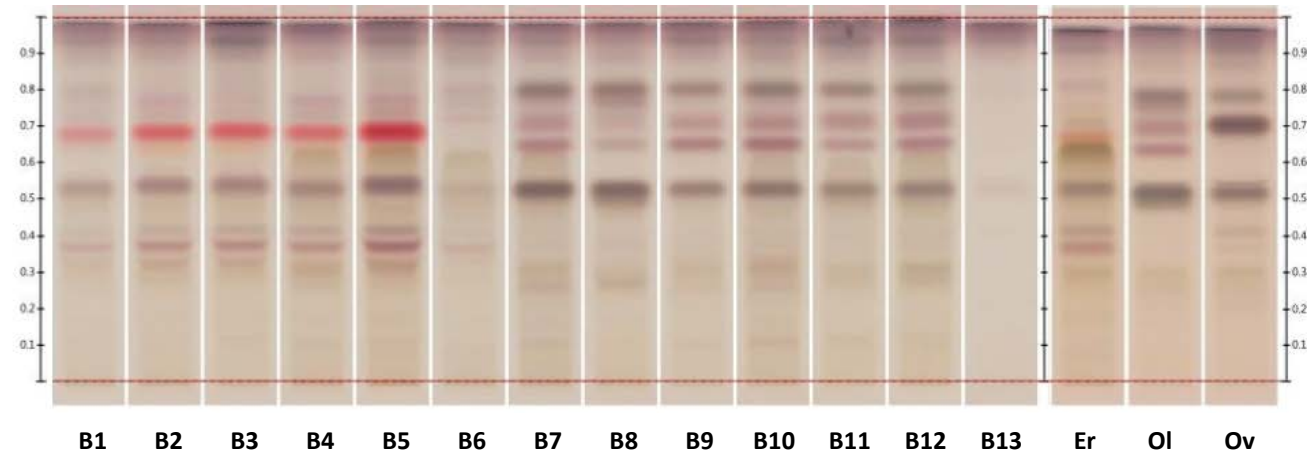
HPTLC Investigation of Commercial Eyebright Samples

- 28 botanical samples collected in the wild, including 25 *Euphrasia* spp. samples, *Odontites lutea*, *O. viscosus*, and *Bartsia alpina*
- 32 commercial samples analyzed: 25 bulk materials and 7 finished products (USA: 28; Europe: 4)
- Bulk materials originating in Bulgaria, Croatia, Macedonia, Poland, and Ukraine



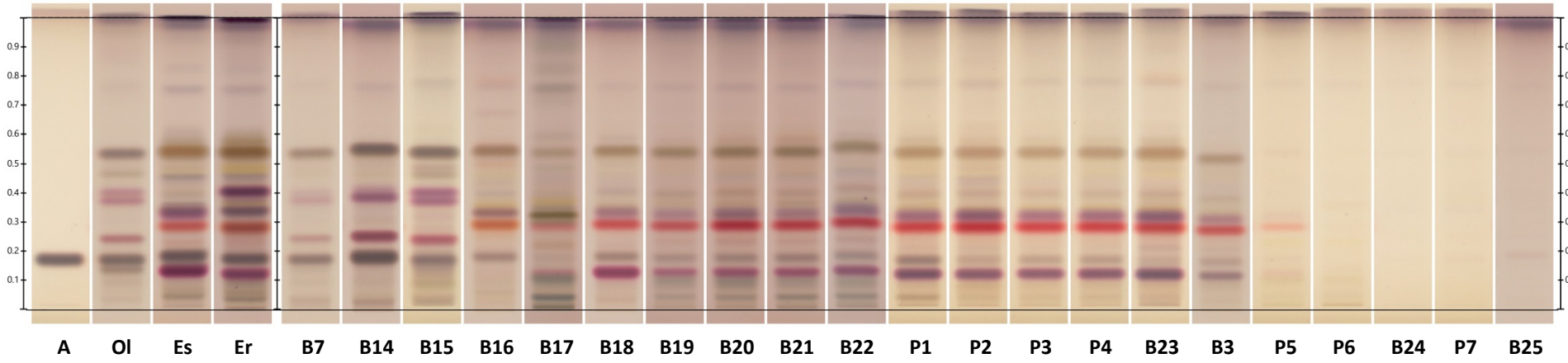
Results

Mobile phase: Dichloromethane, methanol, water (60:40:4)



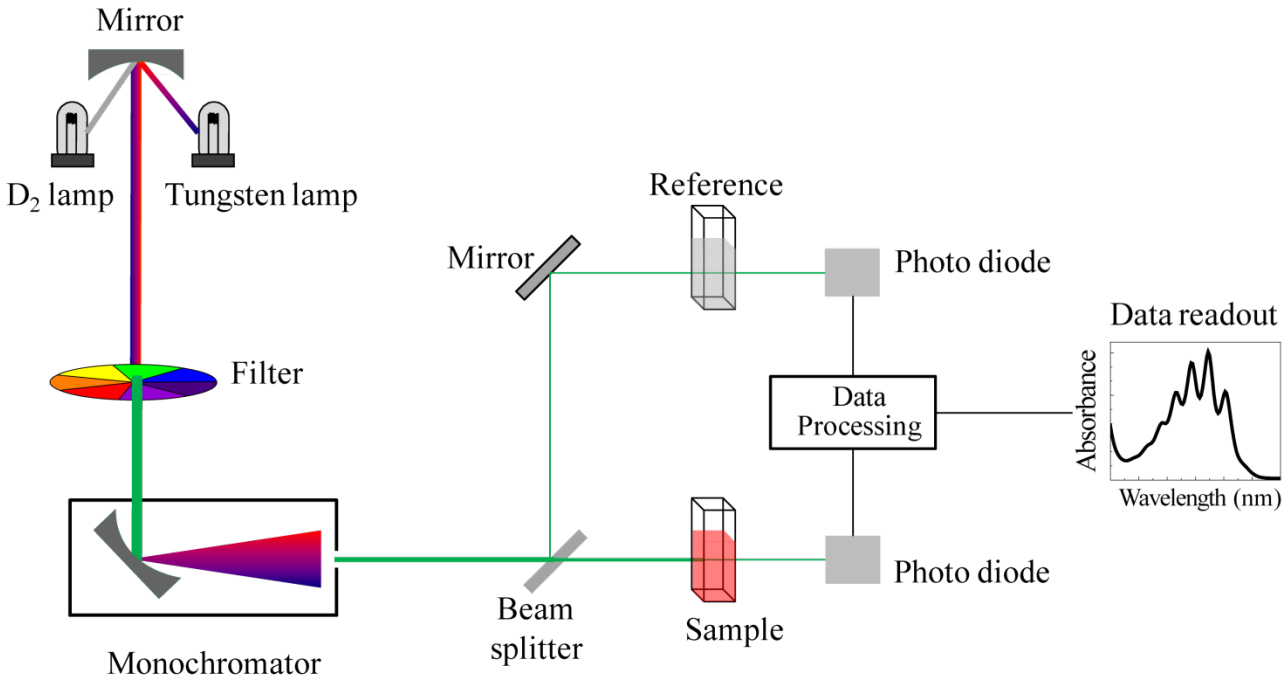
Samples:
B1-B25: Bulk samples
P1-P7: Dietary supplements
A: Aucubin
Er: *Euphrasia rostkoviana*
Es: *Euphrasia stricta*
Ol: *Odontites lutea*
Ov: *Odontites viscosus*

Mobile phase: Ethyl acetate, acetic acid, formic acid, water (100:11:11:26)



Bulk materials: 8 *Odontites* spp. (all from USA), 13 *Euphrasia* spp., 4 weak or blank
Dietary supplements: 4 *Euphrasia*, 3 weak or blank

UV/Vis Spectrophotometry



Absorption at specified wavelength is not specific to an individual marker compound

Applications:

- Total anthocyanins in bilberry, blueberry, elderberry, etc.
- Hypericin content in St. John's wort
- Total proanthocyanidin content in cranberry, grape seed, etc.

AMERICAN BOTANICAL COUNCIL
SINCE 1988

Cranberry: Supply Chain

Cutting of fruit

Pumping berries and water

Filling truck

Removing stems

Sugar-infused cranberry ready for drying

Juice press

Adulteration of Cranberry (*Vaccinium macrocarpon*) Extract

- In 2017, cranberry is the third best-selling herb in the US mass market and # 13 in the US natural channel with sales over US$ 75 Mio*
- Beneficial effects for preventing urinary tract infection reportedly due to proanthocyanidins (PACs), in particular A-type
- Cranberries at 100 g fresh weight provide ca. 420 mg total flavan-3-ols, of which 56% (235 mg) are polymers**
- Other plants contain higher amounts of PACs and present cheaper sources


Vaccinium macrocarpon
Image courtesy of Steven Foster

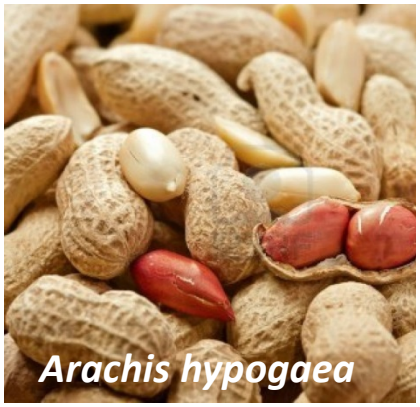
*Smith T, email communication June 19, 2018.

***USDA database for proanthocyanidin content of selected foods. Beltsville (MD): Nutrient Data Laboratory, Agricultural Research Service, USDA 2004.*

Adulteration of Cranberry Extract

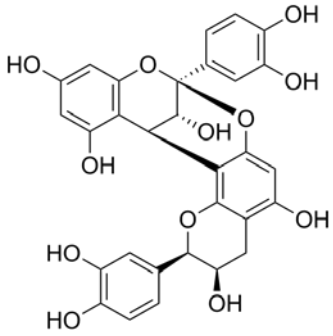
B-type

B-type

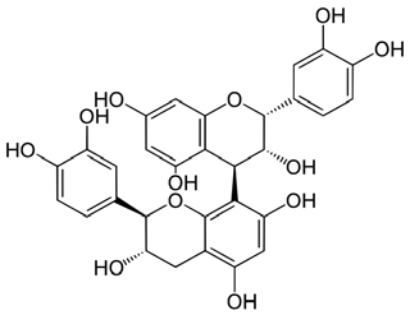


A-type and B-type

A-type and B-type



Procyanidin A2



Procyanidin B1

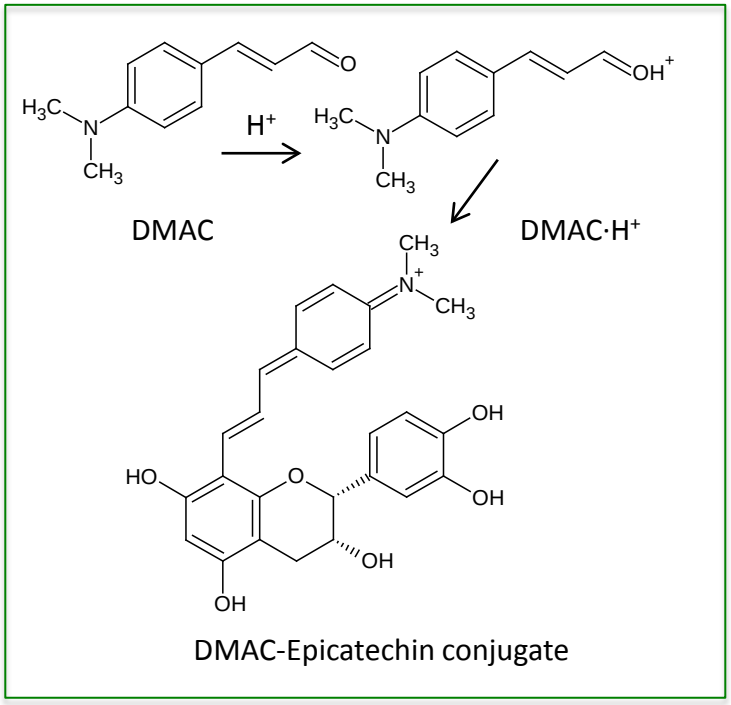
Proanthocyanidins: Complex mixtures – polymers tend to elute as "mole hills" with HPLC

ABC AHP NCNPR
Botanical Adulterants Prevention Program

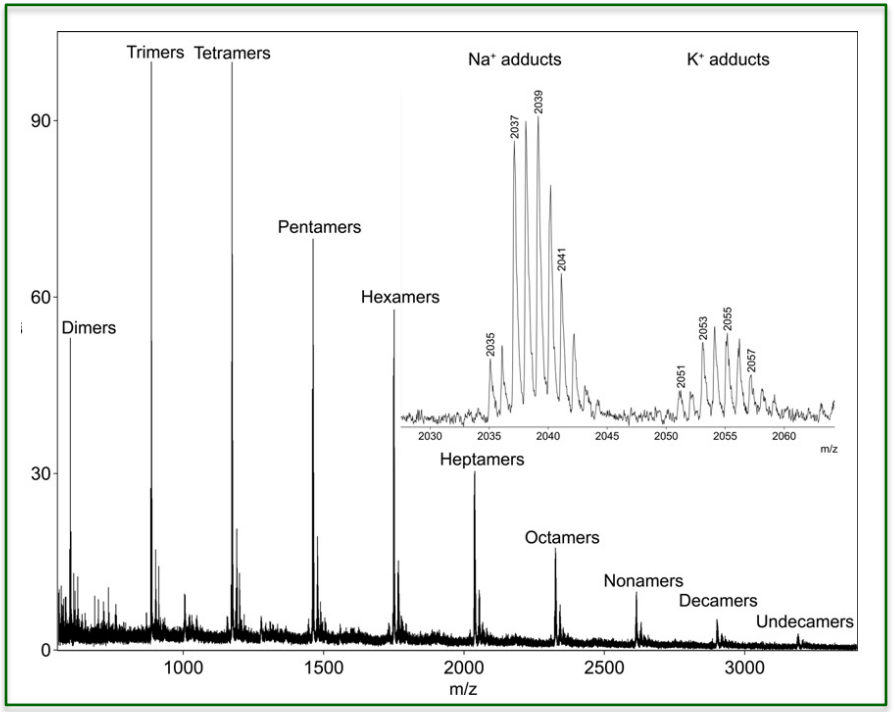
American Botanical Council
25 Years of Herbal Excellence

Specificity: Cranberry Extract

UV/Vis spectrophotometry (DMAC)



MALDI-TOF



MALDI-TOF spectrum of cranberry PACs (Feliciano et al., *Food Chem*. 2012; 135: 1485-1493)

American Botanical Council 25 Years of Herbal Excellence

Botanical Adulterants Program ABC AHP NCNPR

Adulteration of Cranberry (*Vaccinium macrocarpon*)

cranberry juice extract

English name: bilberry extract; cranberry extract
Latin Name: Vaccinium vitis-idaea Linn.
CAS No.: 84082-34-8
Molecular forula:C27H31O16
Molecular Weight: 611.53
Active ingredients: Anthocyanidin
Specification: 10%, 15%, 20%, 25%
Use Part : fruit
Appearance: Dark purple fine powder
Mesh size: 80 Mesh
Test Method: UV

more discount

n stock

uct quality protection

eport Suspicious Activity

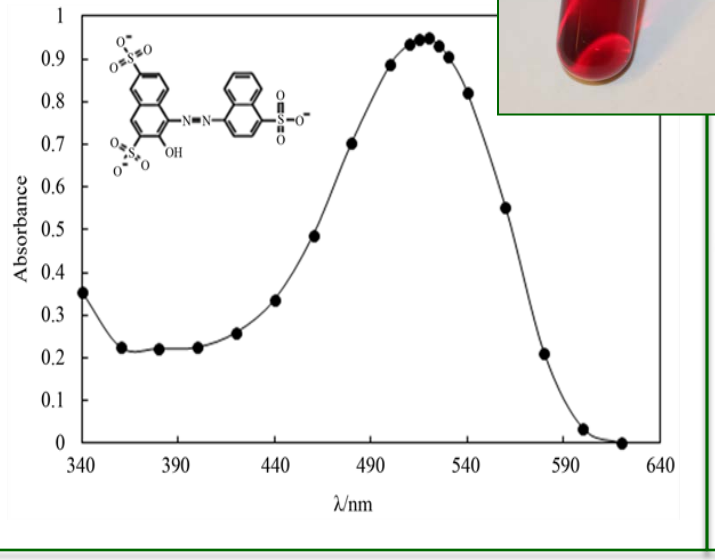
tainer

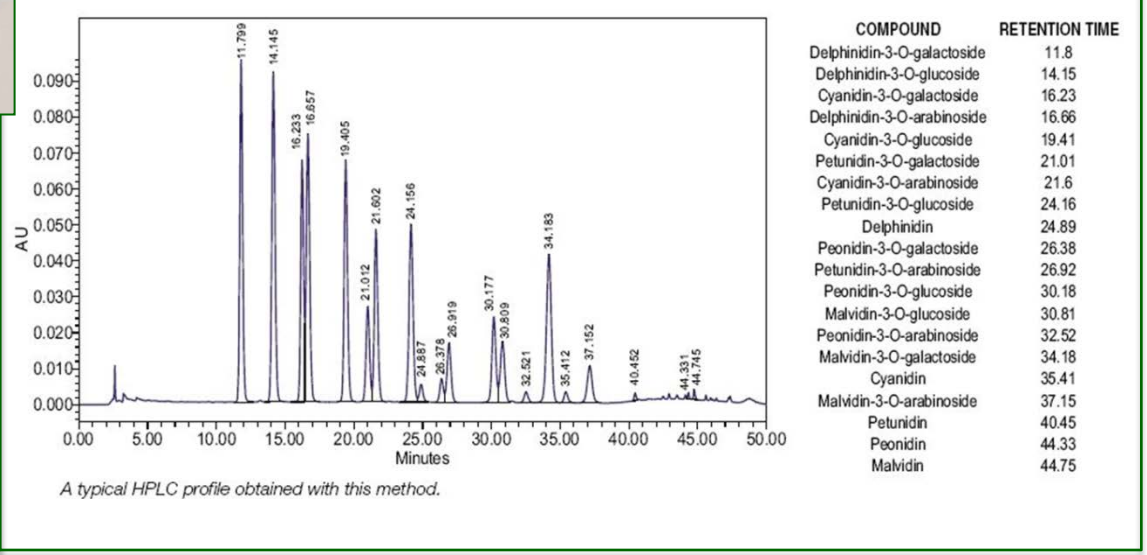
Product Details

Quick Details

Type: Herba
Part: Fruit
Place of Origin: Shaa
Model Number: 25%

Specificity: Bilberry Extract





COMPOUND	RETENTION TIME
Delphinidin-3-O-galactoside	11.8
Delphinidin-3-O-glucoside	14.15
Cyanidin-3-O-galactoside	16.23
Delphinidin-3-O-arabinoside	16.66
Cyanidin-3-O-glucoside	19.41
Petunidin-3-O-galactoside	21.01
Cyanidin-3-O-arabinoside	21.6
Petunidin-3-O-glucoside	24.16
Delphinidin	24.89
Peonidin-3-O-galactoside	26.38
Petunidin-3-O-arabinoside	26.92
Peonidin-3-O-glucoside	30.18
Malvidin-3-O-glucoside	30.81
Peonidin-3-O-arabinoside	32.52
Malvidin-3-O-galactoside	34.18
Cyanidin	35.41
Malvidin-3-O-arabinoside	37.15
Petunidin	40.45
Peonidin	44.33
Malvidin	44.75

UV/Vis spectrum of amaranth dye

HPLC-UV chromatogram (535 nm) of bilberry extract according to the *European Pharmacopoeia*. Image provided by Indena S.p.A. (Milan, Italy).

AMERICAN BOTANICAL COUNCIL
25 YEARS of Herbal Excellence

Botanical Adulterants Program
ABC AHP NCNPR

Bilberry Extract Adulterants

Anthocyanidin-rich extracts from

- Bog bilberry (*Vaccinium uliginosum*)
- Lingonberry (*V. vitis-idaea*)
- Blueberry species (*V. angustifolium, V. corymbosum*)
- Cranberry (*V. oxycoccos and V. macrocarpon*)
- Raspberries (*Rubus* spp., Rosaceae)
- Wild cherry (*Prunus avium*, Rosaceae)
- Black chokeberry (*Aronia melanocarpa*, Rosaceae)
- Mulberry species (*Morus australis, M. nigra*, Moraceae)
- European elder (*Sambucus nigra*, Adoxaceae)
- Black soybean (*Glycine max*, Fabaceae)
- Black rice (*Oryza sativa*, Poaceae)
- Amaranth dye
- Charcoal

Same issue with…

Sambucus nigra subsp. *canadensis*


Aronia melanocarpa
(Image provided by Gayle Engels)


Euterpe oleracea
(www.tradewindsfruit.com)

Botanical Adulterants Program

AMERICAN BOTANICAL COUNCIL
SINCE 1988

GC-FID

Applications: Analysis of volatile compounds (compounds of higher polarity after derivatization)

Potential issues with GC-FID method:

- Use of marker compound(s) that are not be specific for plant/plant part
- Adulterant may not be soluble in solvent used for sample preparation
- Adulterant may not be volatile

Common ways to fool GC-FID:

- Addition of same/similar compounds from other plant sources
- Admixture of nature-identical marker compounds

AMERICAN BOTANICAL COUNCIL
SINCE 1988

Tea Tree Oil

Melaleuca alternifolia

Reported adulterants:

- Waste stream products after rectification of camphor (*Cinnamomum camphora*), eucalyptus (*Eucalyptus* spp.), or pine (*Pinus* spp.) oils
- Monoterpenes obtained via fermentation or semi-synthesis (e.g. catalytic conversion of sabinene to terpinen-4-ol)

Adulteration with terpinen-4-ol may be detected by the presence of *p*-menth-1-ene, *t*-pinocarveol and *p*-menth-3-ene

ABC AHP NCNPR
Botanical Adulterants Prevention Program
American Botanical Council · the American Herbal Pharmacopoeia · the University of Mississippi's National Center for Natural Products Research

Tea Tree Oil Laboratory Guidance Document

By Stefan Gafner, PhD[a]* and Ashley Dowell[b]
[a]American Botanical Council, PO Box 144345, Austin, TX 78714
[b]Southern Cross University, Military Road, East Lismore, NSW 2480, Australia
*Corresponding author: email

Tea Tree *Melaleuca alternifolia*
Photo©2018 Down Under Enterprises

Citation (JAMA style): Gafner S, Dowell A. Tea tree oil laboratory guidance document. Austin, TX: ABC-AHP-NCNPR Botanical Adulterants Prevention Program. 2018.

Keywords: Adulteration, *Eucalyptus globulus*, eucalyptus oil, *Melaleuca alternifolia*, *Melaleuca linariifolia*, tea tree oil, white camphor oil

1. Purpose

Tea tree oil (TTO) is the essential oil of tea tree (*Melaleuca alternifolia* or *M. linariifolia*, Myrtaceae). Adulteration of TTO has become more apparent in recent years. Adulteration occurs with single essential oil components (e.g., sabinene from pine oil), waste products derived from other essential oils such as pine (*Pinus* spp., Pinaceae), eucalyptus (*Eucalyptus*

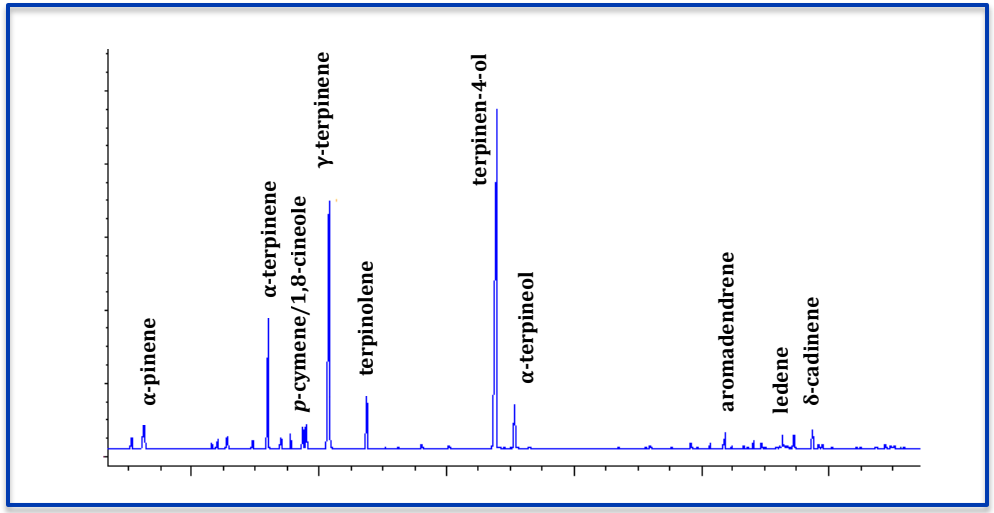
Tea Tree Oil Laboratory Guidance Document

Detailed chemical composition of TTO and its adulterants

Assessment of:

- Macroscopic, microscopic, and organoleptic tests
- Genetic assays
- Physicochemical tests
- Chemical Analysis
  - = HPTLC
  - = Infrared (IR, MIR, NIR)
  - = Gas Chromatography

Input from 13 peer-reviewers



GC-FID chromatogram of authentic tea tree oil, terpinen-4-ol type. Conditions according to Southwell and Russell, *Phytochemistry.* 2002;59(4):391-393.

HPLC-UV/Vis

Applications: Almost any botanical ingredient

Potential issues HPLC-UV/Vis method:

- Use of marker compound(s) that are not be specific for plant/plant part
- Adulterant may not be soluble in solvent used for sample preparation
- Adulterant lacks chromophore
- Highly polar adulterant

Common ways to fool HPLC-UV/Vis:

- Addition of same/similar compounds from other plant sources
- Addition of extracts from other plant parts
- Admixture of nature-identical marker compounds

AMERICAN BOTANICAL COUNCIL
SINCE 1988

ABC AHP NCNPR
Botanical Adulterants Prevention Program
BOTANICAL ADULTERANTS BULLETIN

Adulteration of *Ginkgo biloba* Leaf Extract

By Stefan Gafner, PhD*

American Botanical Council, 6200 Manor Road, Austin, TX 78723

**Corresponding author:* email

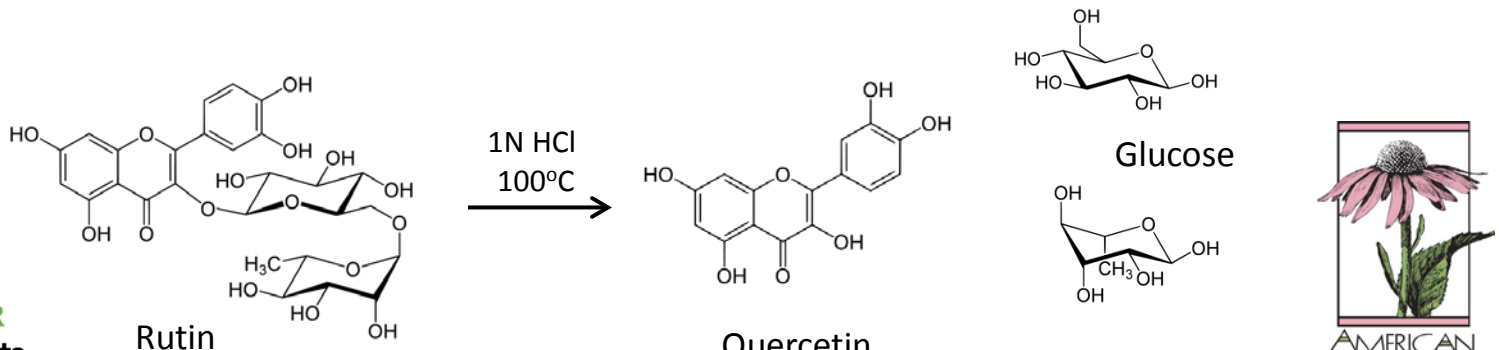
Keywords: adulterant, adulteration, *Ginkgo biloba*, ginkgo leaf extract, Japanese pagoda tree, Japanese sophora, kaempferol, quercetin, rutin, *Sophora japonica*, *Styphnolobium japonicum*

Goal: The goal of this bulletin is to provide timely information and/or updates on issues of adulteration of ginkgo (*Ginkgo biloba*) leaf and ginkgo leaf extracts to the international herbal industry and extended natural products community in general. It is intended to give a brief overview on the occurrence of adulteration, known adulterants and analytical means to detect them, the market situation,

Pharmacopeial (USP) Method to Determine Total Flavonol Glycosides in Ginkgo Extracts

- Quercetin-, keampferol-, and isorhamnetin-glycosides are subjected to hydrolysis
- Resulting quercetin, keampferol, and isorhamnetin are quantified by HPLC-UV
- Total flavonol glycosides are quantified using conversion factor (2.504 for quercetin, 2.588 for kaempferol, and 2.437 for isorhamnetin)

- Adulteration by addition of pure quercetin, kaempferol, or rutin, or rutin-rich extracts from other sources (buckwheat, Japanese sophora)



ABC AHP NCNPR
Botanical Adulterants Prevention Program

AMERICAN BOTANICAL COUNCIL
SINCE 1988

Adulteration of Ginkgo biloba extracts

Summary of 21 investigations into the authenticity of commercial extracts from 2003-2019

	Sample number	Adulterated samples	Adulteration [%]
All samples*	418	192	45.9
Asia	35	6	17.1
Australia/New Zealand	14	6	42.9
Europe	174	88	50.6
Europe (herbal medicine)*	18	0	0
Europe (food supplement)*	85	49	55.3
North America	69	37	53.6

*Some authors did not detail the origin of the analyzed products, or distinguish among food supplements and herbal medicine

Botanical Adulterants Program

ABC AHP NCNPR

AMERICAN BOTANICAL COUNCIL

SINCE 1988

Same issue with…


Crataegus spp.


Passiflora incarnata

Botanical Adulterants Program

ABC AHP NCNPR

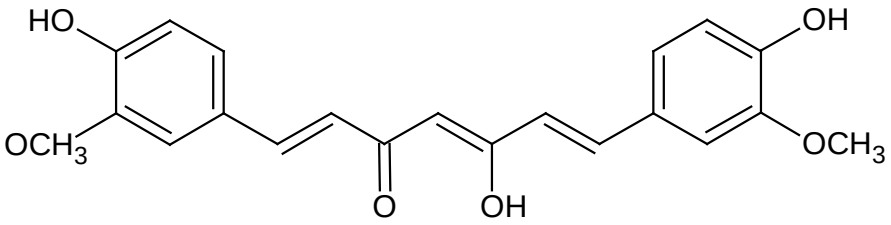
AMERICAN BOTANICAL COUNCIL

SINCE 1988

Adulteration of Turmeric Root/Rhizome and Extracts

- Turmeric extracts contain diarylheptanoids with a plethora of reported health benefits
- Best-selling herbal dietary supplement in Natural channel, 5th in mass market in U.S. with sales above US $ 80 million*
- Main use is to treat inflammatory conditions

*Smith T, Kawa K, Eckl V, Morton C, Stredney R. *HerbalGram*. 2018;103:62-71.



Curcumin

Curcuma spp.

Adulteration of Turmeric Root/Rhizome and Extracts

- Dried turmeric has a rough surface and dull color: roots are often polished to look more appealing
- After polishing, visual aspect of roots may be improved by wet or dry coloring (turmeric powder, undeclared synthetic colorants, e.g. lead chromate, metanil yellow)
- Other *Curcuma* species may be used as substituents
- Synthetic curcumin added to comply with standardization requirements

Curcuma longa

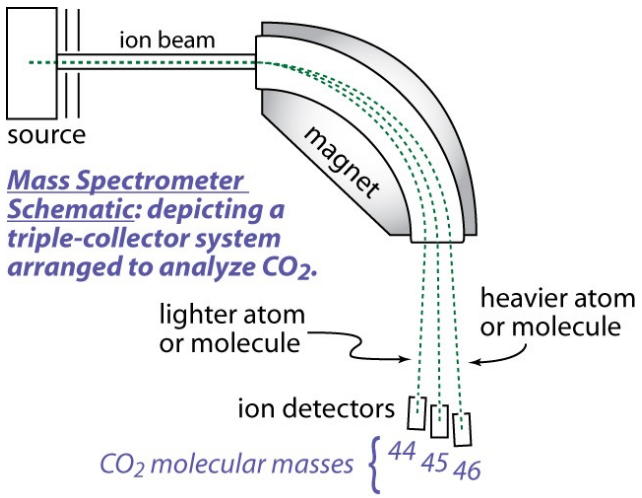
Curcuma zedoaria

Curcuma zanthorrhiza

Detection: Carbon Isotope Measurements

- Steady-state between incorporation of ^{14}C (via photosynthesis using CO_2) into plant and its decay (half-life of 5730 years)
- After plant dies, no further incorporation of ^{14}C
    - Concentration of ^{14}C is highest in living plants
    - Fossil fuel-derived products have little or no ^{14}C

- Certain plants (grasses, corn, sugar cane) incorporate ^{13}C at lesser amounts than others
    - $^{13}C/^{12}C$ ratio can be used to determine corn– or sugar cane-derived materials



Is Synthetic Curcumin/Piperine Involved in Italian Hepatitis Outbreak?

il fatto alimentare

Home | Sicurezza Alimentare | Etichette & Prodotti | Pubblicità & Bufale | Nutrizione | Pianeta | Recensioni & Eventi

Home / Allerta /

Curcuma ed epatite: salgono a 21 i casi individuati. Segnalati 19 diversi integratori alimentari, ma ancora nessuna spiegazione

Giulia Crepaldi 27 Giugno 2019 Allerta 1 Commento

Continuano le segnalazioni di casi di epatite colestatica acuta, un'infiammazione del fegato che non è infettiva né contagiosa, e con esse quelle degli integratori alimentari a base di curcuma e curcumina. Al 20 giugno, erano 21 le persone colpite individuate dall'Istituto superiore di sanità.

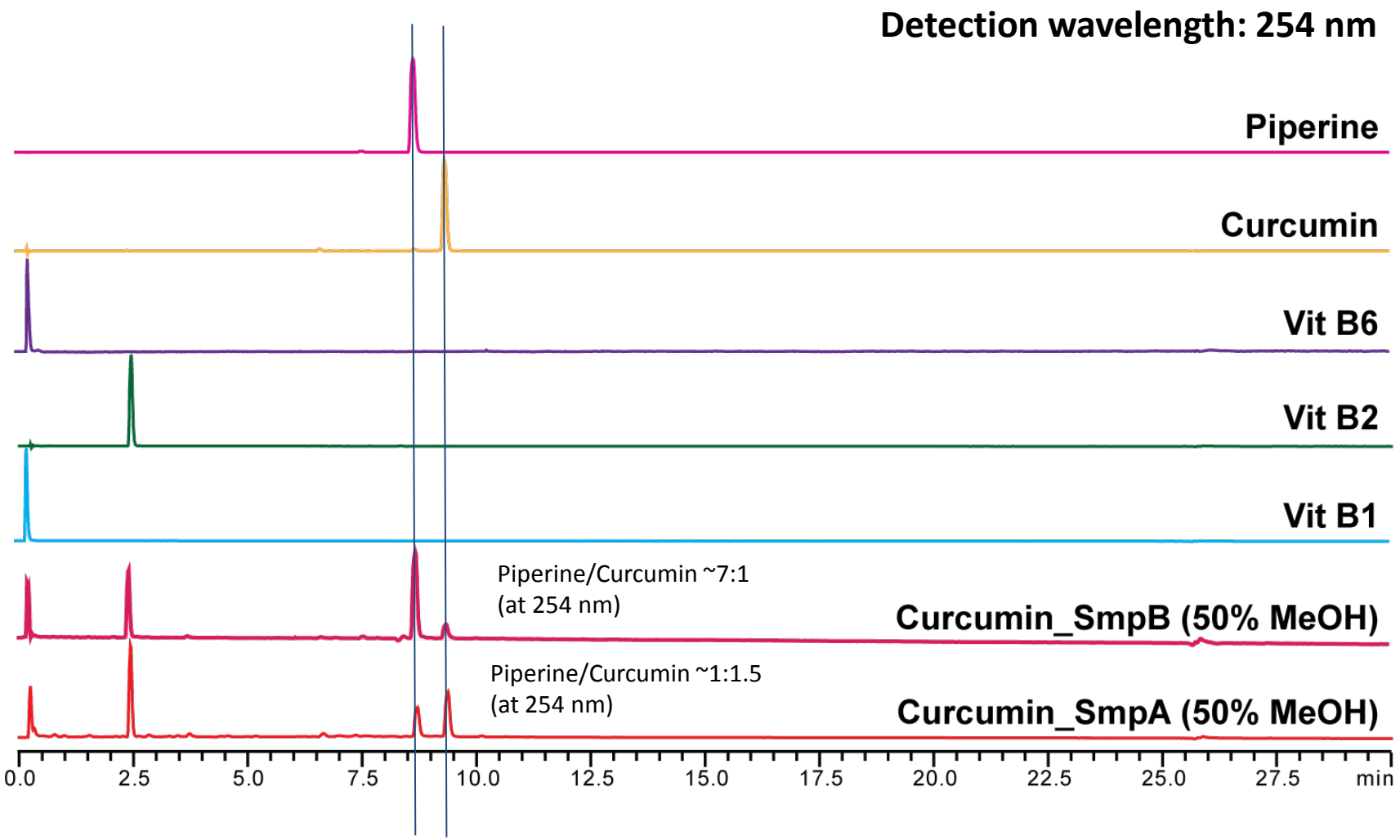
Iscriviti alla newsletter

Email: *

Nome: *

Cognome: *

UHPLC Analysis of 50% MeOH Extracts of Batches A+B
Reference Standards: Curcumin, Piperine, and Vitamins (B1, B2, and B6)



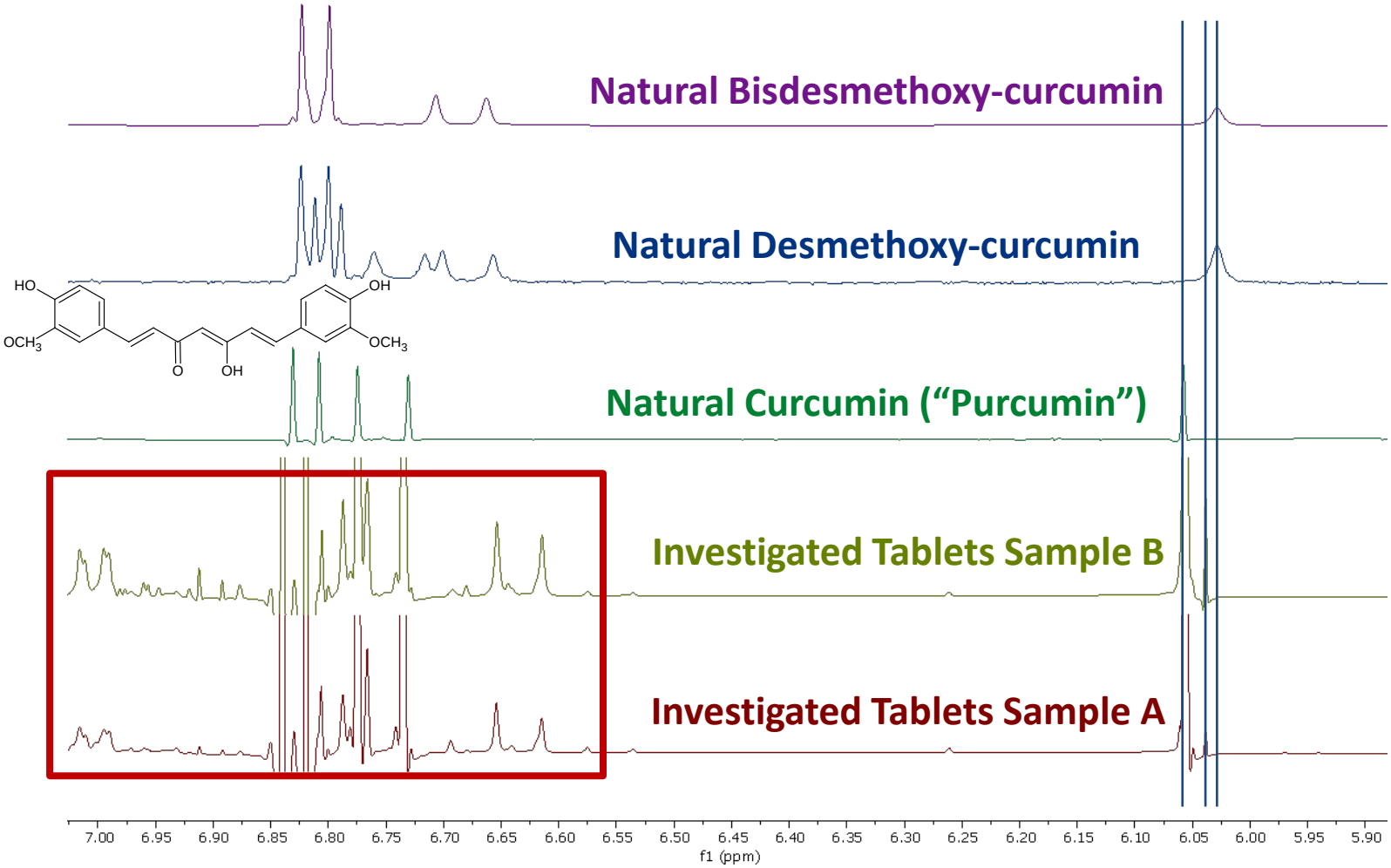
Net relative ratio piperine/curcumin ~11-fold ratio difference (at 254 nm)

Image provided by Guido Pauli, University of Illinois at Chicago

(q)HNMR Analysis of Samples A and B, aka "Yellow Tablets"

S-B Kim JB Friesen S-N Chen GF Pauli CENAPT@University of Illinois at Chicago

- "Yellow tablets" do **not** contain natural desmethoxy-curcuminoids
- "Yellow tablets" contain **undeclared aromatic component**



Acknowledgements

- Mark Blumenthal
- Ezra Bejar
- John Cardellina
- Steven Foster
- Ikhlas Khan
- Roy Upton
- ABC staff
- Débora Frommenwiler
- Frank Gafner
- Ernesto Martinelli
- Roberto Pace
- Guido Pauli
- Eike Reich
- Charly Rey

>200 ABC-AHP-NCNPR Botanical Adulterants Program Underwriters & Endorsers

ABC AHP NCNPR
Botanical Adulterants Prevention Program

AMERICAN BOTANICAL COUNCIL
25 YEARS
of Herbal Excellence