THE UNIVERSITY of MISSISSIPPI

Classification of Chamomile Flowers, Essential Oils and Commercial Products Utilizing Chemometrics and the Agilent GC/MSD

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What Is Chamomile?

Chamomile: one name, different botanical species

German Chamomile

Matricaria chamomilla L.

Roman Chamomile

Chamaemelum nobile (L.) All.

Juhua

Chrysanthemum morifolium Ramat.

All chamomile species belong to the same *Asteraceae (Compositae)* family but different genera

Why Is Chamomile Important?

Medicinal Properties

- Anti-inflammatory
- Hay fever
- Wound healing and burn relief
- Gastro-intestinal disorders
- Tooth ache, ear ache
- Ulcers
- Antibacterial
- Antifungal
- Infections
- Common cold
- Laryngitis
- Anxiety relief
- Sleep disorders
- Cardiovascular diseases
- Muscle spasm
- Rheumatic pain
- Arthritis

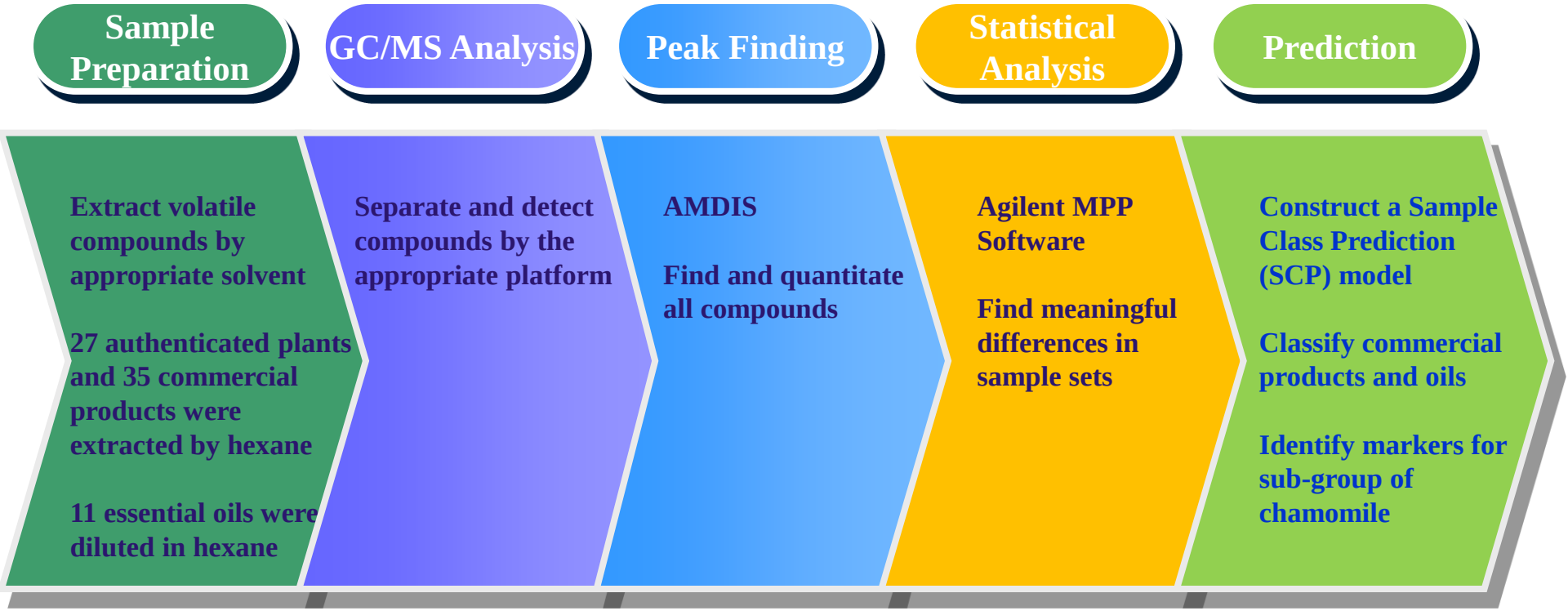
Commercial Products

- Cosmetics
- Aromatherapy
- Teas
- Lotions
- Herbal Beer
- Gargles
- Shampoos

Why Did We Study Chamomile?

- Many products, few clinical studies on humans
- Reported adverse reaction, allergies, skin rash, throat swelling, drowsiness and anaphylaxis
- Poor definition of chamomile
- Potential safety issues with commercial products and dietary supplements
- Detection of adulteration/substitution in commercial products

What Workflow Did We Use?



How Did We Obtain Data?

GC/MS Analysis

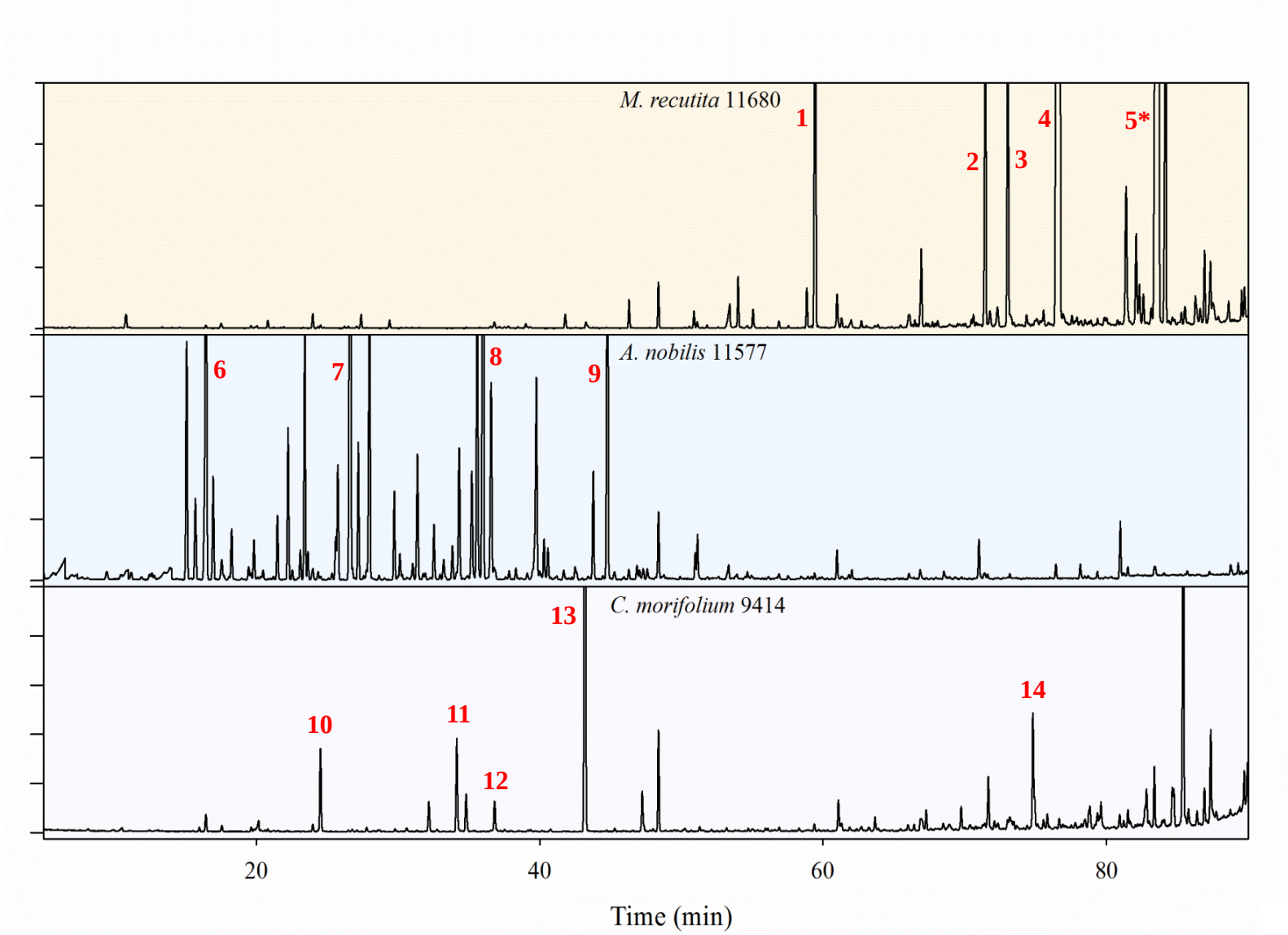
Advantages:

- GC resolves complex samples
- Ionization produced by collision with 70 eV electrons
- Fragmentation pattern characteristic of molecular structure
- **Many libraries available**
- GC/QToF instrumentation gives 2-5 ppm accuracy in *m/z*.
- Selected ion monitoring GC/QQQ allows very accurate quantitation at high sensitivity

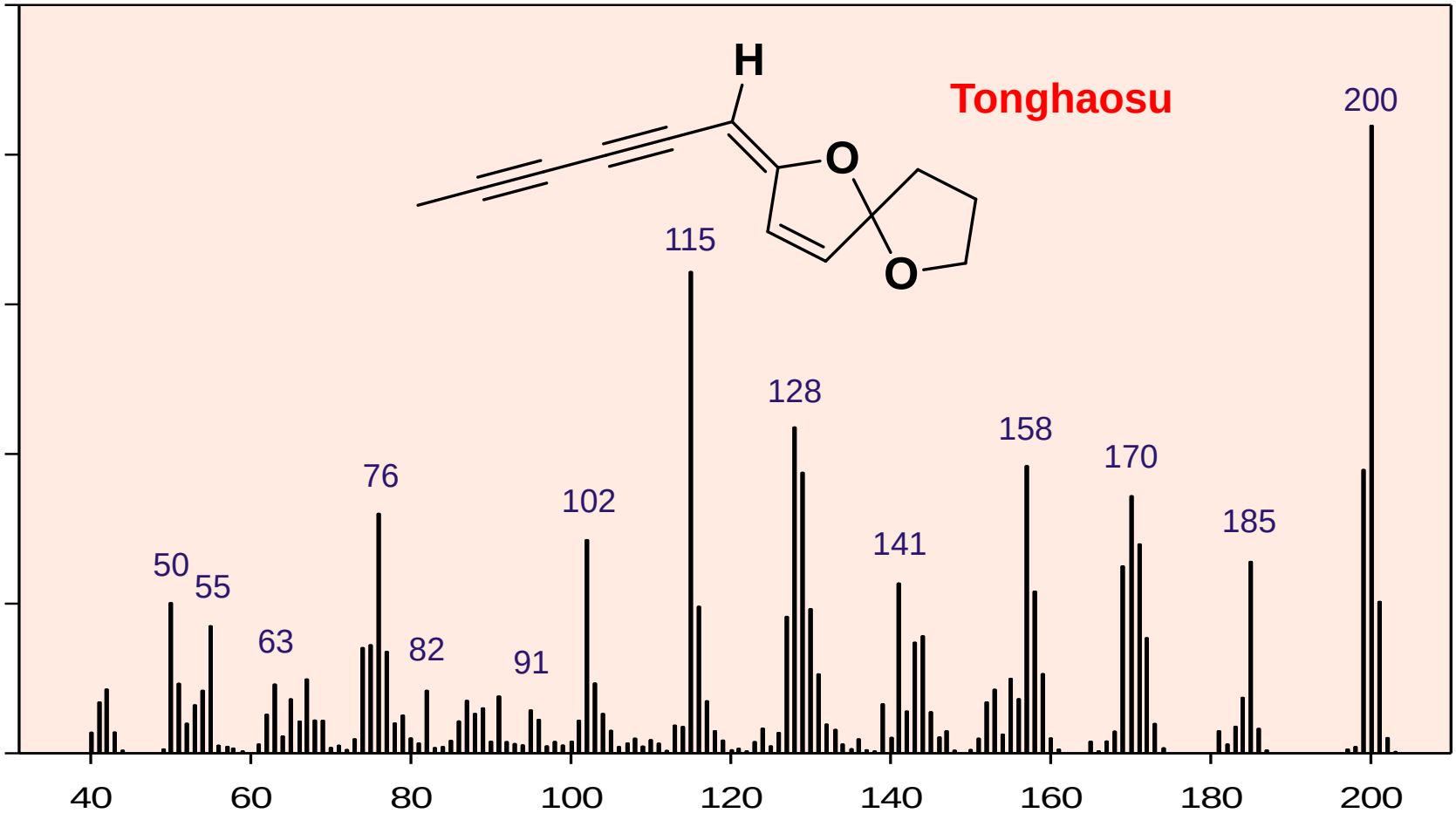
Disadvantages:

- Sample must be volatile (300 ºC)
- Complex and expensive instrumentation
- Fragmentation sometimes destroys molecular ion.

What Can We Get From GC?

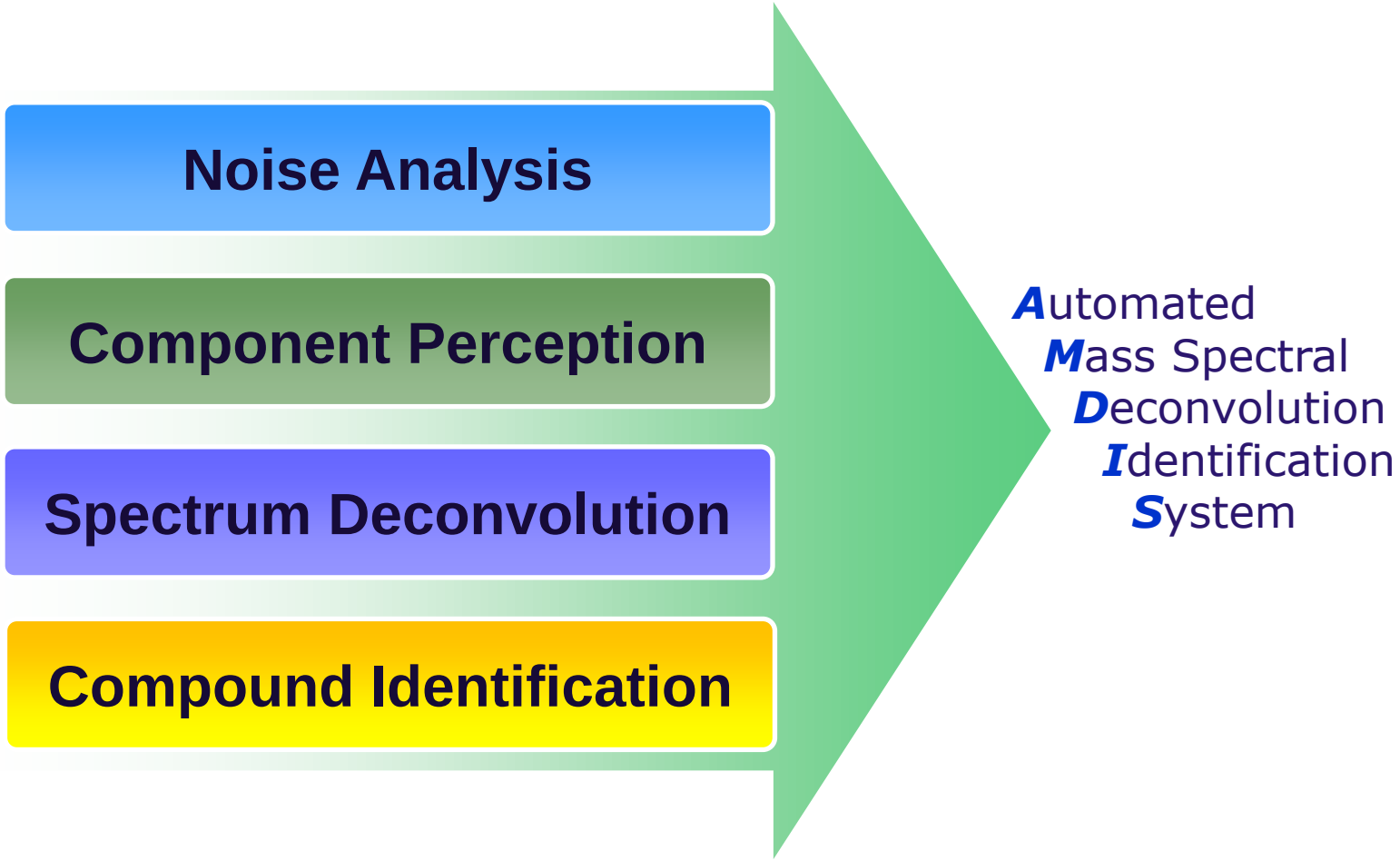


What Can We Get From MS?



How Did We Analyze Data?

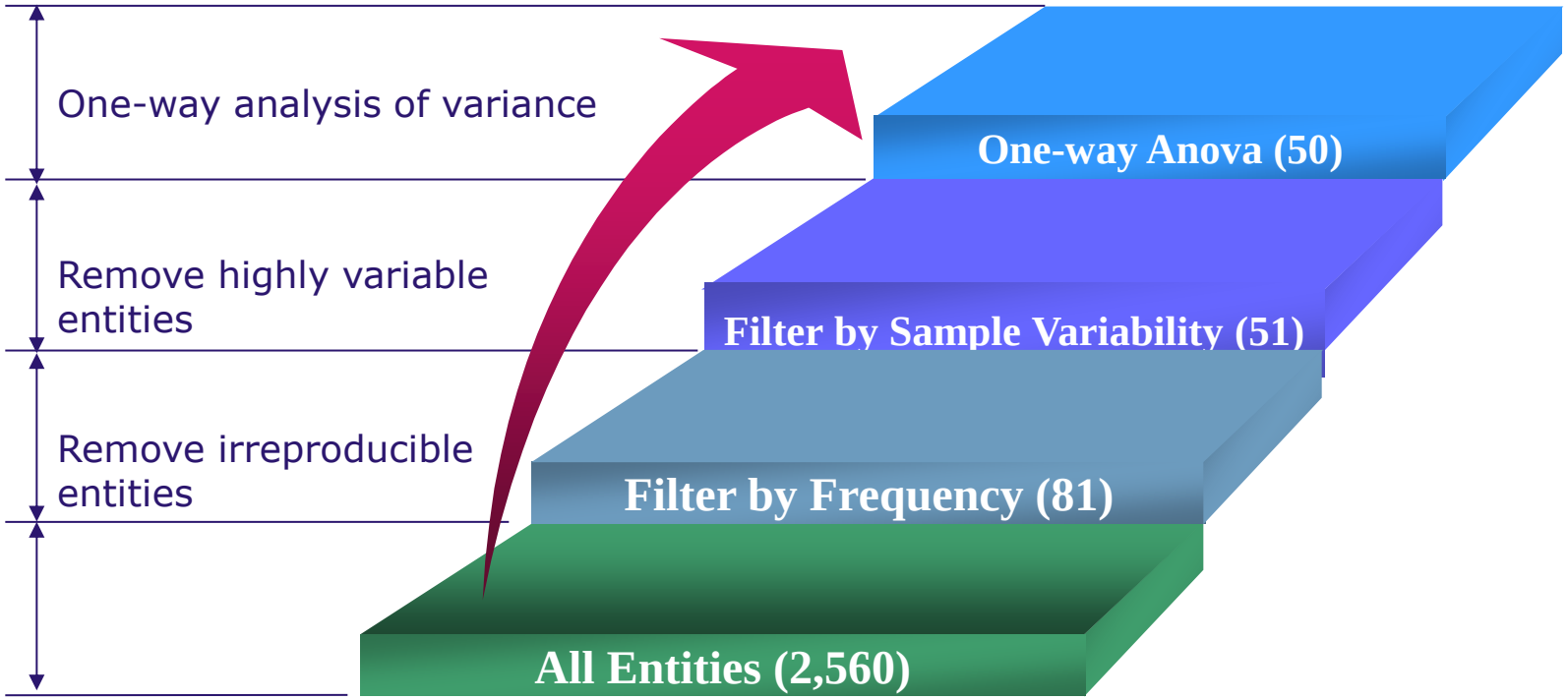
Preparation of Data for Statistical Analysis Using *AMDIS*



AMDIS automatically extracts pure (background free) component mass spectra from highly complex GC-MS data files and uses these purified spectra for further statistical analysis

How Did We Reduce Data?

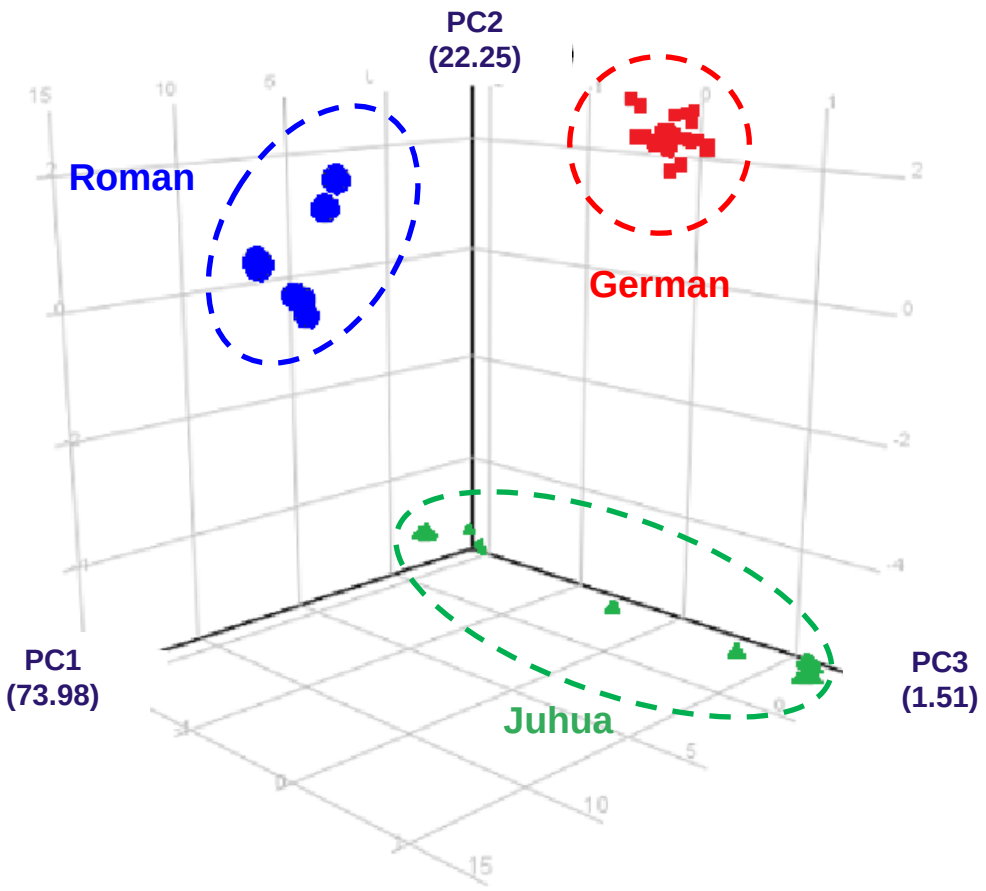
Find Meaningful Differences in Sample Sets Using Agilent *Mass Profiler Professional*



- To explore the most characteristic markers representing different chamomiles
- To reduce the dimensionality of the data

What Did We Do With PCA?

Principal Component Analysis (PCA)



Uses for:

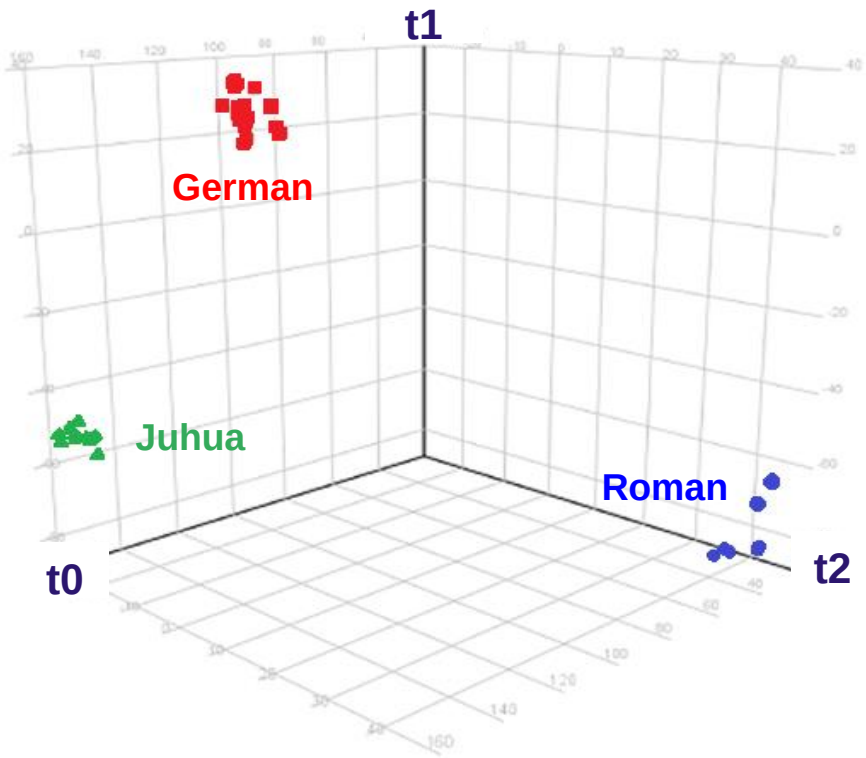
- Data Visualization
- Data Reduction
- Data Classification
- Trend Analysis

Solved Problems in the Study:

- How many unique “sub-sets” are in the samples?
- How are they similar / different?
- What are the underlying factors that influence the samples?
- Which measurements are needed to differentiate?

How Did We Constructed SCP Model?

Sample Class Prediction (SCP) Model –
Partial Least Squares Analysis (PLS-DA)



	German	Roman	Juhua	Accuracy (%)
Model Training				
German	15	0	0	100.0%
Roman	0	4	0	100.0%
Juhua	0	0	8	100.0%
Recognition Ability (%)				100.0%
Model Validation				
German	4	0	0	100.0%
Roman	0	4	0	100.0%
Juhua	0	0	4	100.0%
Prediction Ability (%)				100.0%

How Did We Use SCP Model?

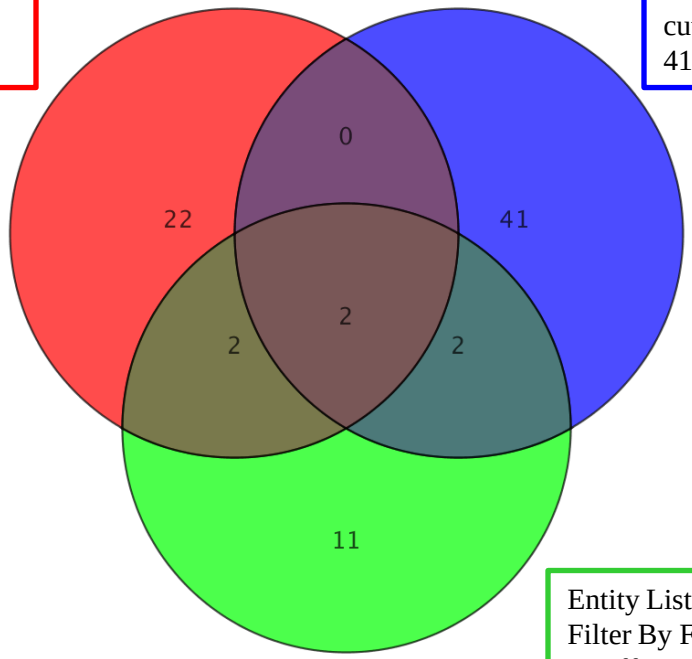
NCNPR Accession Code	Product Information from the Label	Predicted	Confidence Measure
2061	Roman chamomile	German	0.47
3670	Chamomile flower	German	0.92
3998	Chamomile extracts	German	0.53
4903	Chamomile powder	German	0.90
5770	Chamomile powder	German	0.93
9357	Chamomile flowers	German	0.82
9359	Chamomile flowers	German	0.84
9362	Chamomile flowers	German	0.84
9364	Chamomile flowers	German	0.92
9382	Chamomile Organic Tea (Leaves and flowers)	German	0.94
9383	Herbal Chamomile & Fruit Tea (Rosehips, chamomile, orange peel, lemon peel & lemon myrtle)	German	0.72
9384	Chamomile Herb Tea	German	0.58
9385	Organic Tea	German	0.81
9386	Carrington Tea-Chamomile	German	0.75
9387	Chamomile Herbal Tea	German	0.91
9388	Chamomile Herb Dietary Supplement	German	0.89
9389	Chamomile Herbal Tea	German	0.61
9390	Chamomile Herbal Tea	German	0.92
9391	Chamomile Herbal Tea	German	0.77
9393	Whole German Chamomile Flowers	German	0.87
9423	Chamomile Herbal Dietary Supplement	Juhua	0.83
9424	Chamomile Herbal Dietary Supplement	Juhua	0.84
9425	Chamomile Herbal Dietary Supplement	Juhua	0.60
9426	Chamomile Herbal Dietary Supplement	Juhua	0.86
9428	Chamomile Herbal Dietary Supplement	Juhua	0.82
9429	Chamomile Herbal Dietary Supplement	Juhua	0.81
9432	Chamomile Herbal Dietary Supplement	Juhua	0.99

How Did We Study Chamomile?

Data Evaluation – Venn Diagram

Entity List 1: German Only
Filter By Frequency with
cutoff percentage: 100.0
22 entities

Entity List 2: Roman Only
Filter By Frequency with
cutoff percentage: 100.0
41 entities



Entity List 3: Juhua Only
Filter By Frequency with
cutoff percentage: 100.0
11 entities

How Did We Study Chamomile?

Markers Identified From Venn Diagram

Entities		Tentative NIST Identification	Molecular Weight	CAS Number
m/z	t_R (min)			
Roman Chamomile				
71.0	15.10	Isobutyric acid, isobutyl ester	144	97-85-8
71.0	23.42	Isobutyric acid, 2-methylbutyl ester	158	2445-69-4
55.0, 83.0	26.64	Butyl Butenoate[a,b]	156	54056-51-8
83.0	39.75	3-Methyl-2-butenoic acid, 3-methylbut-2-enyl ester	168	299309
100.0	44.75	Hexyl Butenoate	324	60129-26-2
German Chamomile				
205.0	66.94	Spathulenol	220	77171-55-2
143.0	71.43	α-Bisabolol oxide B[a,b,c]	238	26184-88-3
93.0, 141.0	73.04	α-Bisabolol[a,b,c]	222	515-69-5
143.0	76.07	Bisabolol oxide A[a,b,c]	238	22567-36-8
128.0	83.70	*E*-1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)-	200	50257-98-2
Juhua				
95.0	36.82	Borneol	154	10385-78-1
132.0	61.06	α-Curcumene	202	644-30-4
91.0	67.27	Caryophyllene oxide	220	1139-30-6
105.0, 121.0	69.75	Alloaromadendrene oxide	220	156128
204.0	71.69	Eudesm-7(11)-en-4-ol	222	473-04-1

What Did We Learn?

- Chemometrics can be used to analyze large, complex (3-D) data sets MUCH faster than manual analysis
- With AUTHENTICATED samples, an accurate sample class prediction model can be developed and verified
- The SCP model can subsequently be used to analyze samples in an automated manner w/o reanalysis of the authenticated samples
- Chemometric analysis can be used to identify potential markers for different type of samples

ACKNOWLEDGEMENTS

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Agilent Application Note

Food Testing & Agriculture

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