

METABOLOMIC APPROACH FOR GREEK HONEY ORIGIN DISCRIMINATION MAKING USE OF ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY



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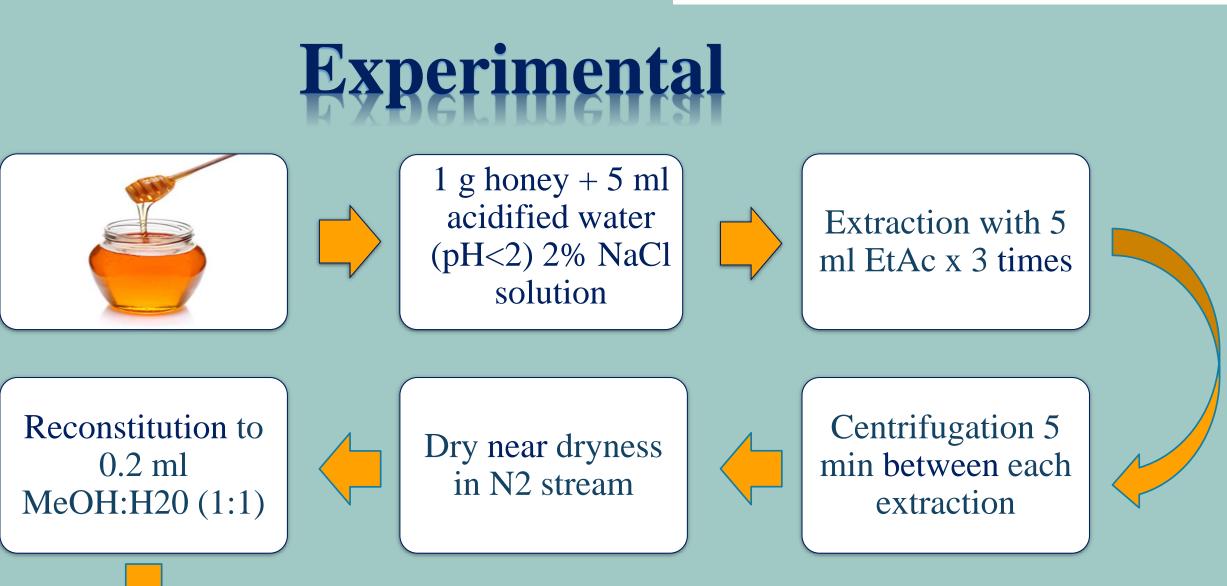


11th International Conference on Instrumental Methods of Analysis Modern Trends and Applications



ABSTRACT

Honey is a foodstuff which is subjected to various deceitful practices, such as addition of syrups or mislabelling due to its high price in the market. Many honeys in the market, sold as unifloral, are often adulterated with others inferior in quality products, on account of great demand and high production cost. It is widely known that honey obtained from specific plants is strictly associated with unique organoleptic and/or health beneficial properties [1]. Hence, the evaluation and verification of honey authenticity is a task of paramount importance for the producers, consumers and regulatory bodies. Untargeted metabolomics using UPLC-ESI-QTOF MS is a powerful approach for the simultaneously analysis of many compounds as well as identify new biomarkers which can discriminate the samples according to their origin. A generic extraction protocol was utilized in order to obtain the whole metabolic profile of the samples. The developed method was applied to 135 Greek honey samples from 5 different botanical origins. Most of the samples are unifloral while some other are polyfloral. The nontarget screening approach was performed using Bruker Metaboscape 3.0 software which incorporates sophisticated tools for profiling, statistical analysis and compound identification. New compounds which differentiate the samples according to botanical and geographical origin were finally identified. The same samples were deeply investigated by a uniquely developed methodology and screening workflow called "AutoSuspect" making use a novel MS-ready database containing hundreds of thousands of naturally occurring compounds [2], and the results were compared.



Data Treatment Metaboscape workflow

T-ReX 3Dalgorithm(Time alignedRegionCompleteeXtractionalgorithm).

- Automated calibration
- de-isotoping algorithm
- retention-time alignment algorithm
- de- adducting
- MS/MS spectrum assigned to each individual Bucket automatically

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	Export		7	253.0 254.	05810 ± 🔤	9.70	C1	H10O4	AL SF		-			Chrysin	-0.00	0.186	0.734	15.
		۲.	8	239.0 240.	07874 ± 🔤	10.10	C19	H ₁₂ O ₃	AL SF	 }	+			2',4'-Dihydroxy	0.00	0.094	0.393	14.
	Save		9	153.0 154.	02654 ± 🔤	1.33	C	7H6O4	AL SF		↓ ● ○			3,4- dihydroxy	0.03	0.033	0.219	8.0 🗸
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M MS/MS Importer		
-	IS/MS Spectra to Buck lerances for assigning a N	tet Assignment IS/MS spectrum to a Bucket.
Configuration		
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Exclusion Criteria Max signal in non-selected analyses		I	ń	I.	1	i 1	- 20
Max signal in selected analyses Include recursive signals	<=	I	Ļ	I	I		3.0
Flag/Exclude Buckets							

Flag Buckets: RED

Exclude buckets: 🔽

Bucket Statistics 🕸 Box Plot 🔗 Chromatograms Mass Calibration

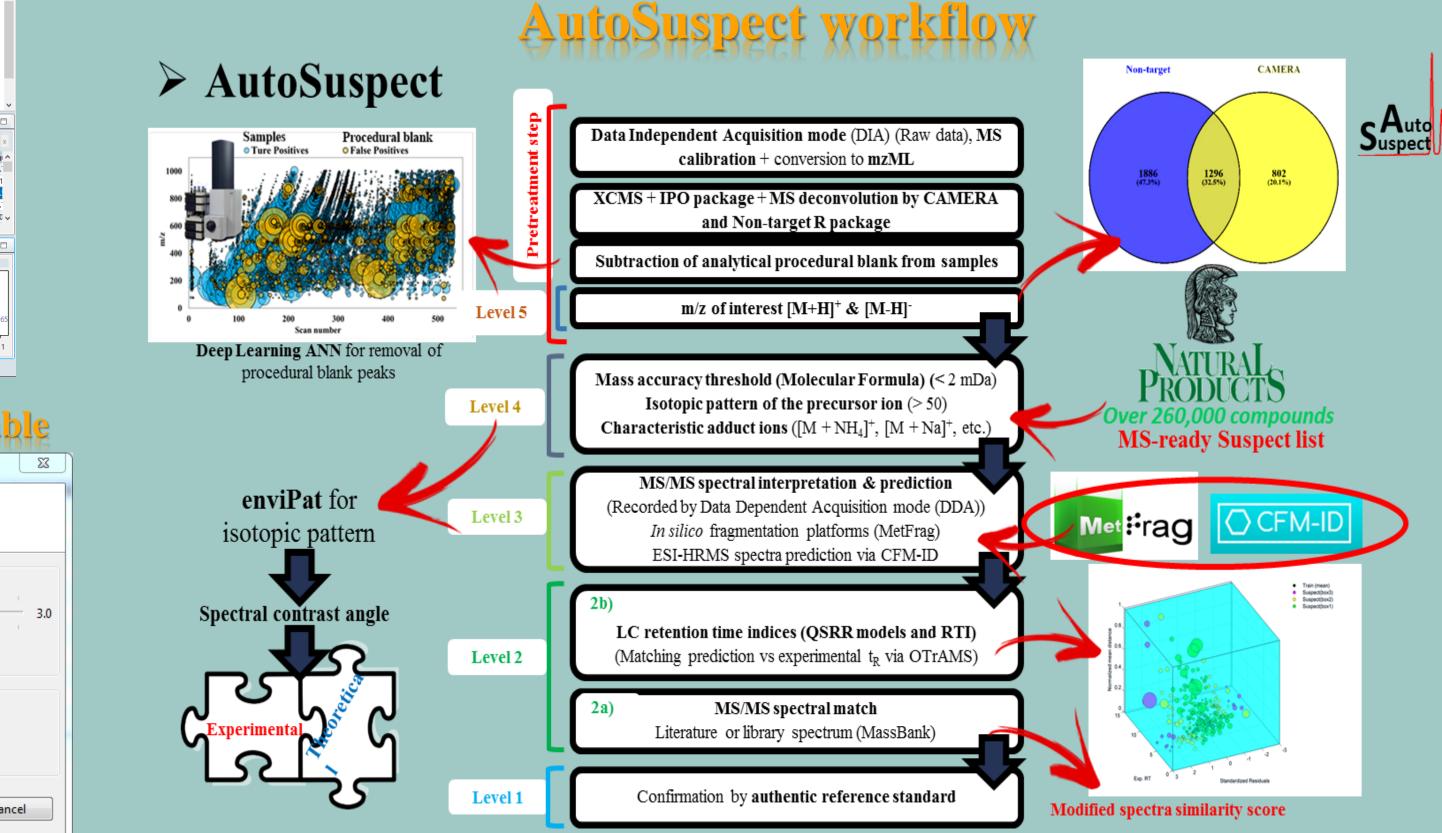
Volcano Plo

\$ 4⊳

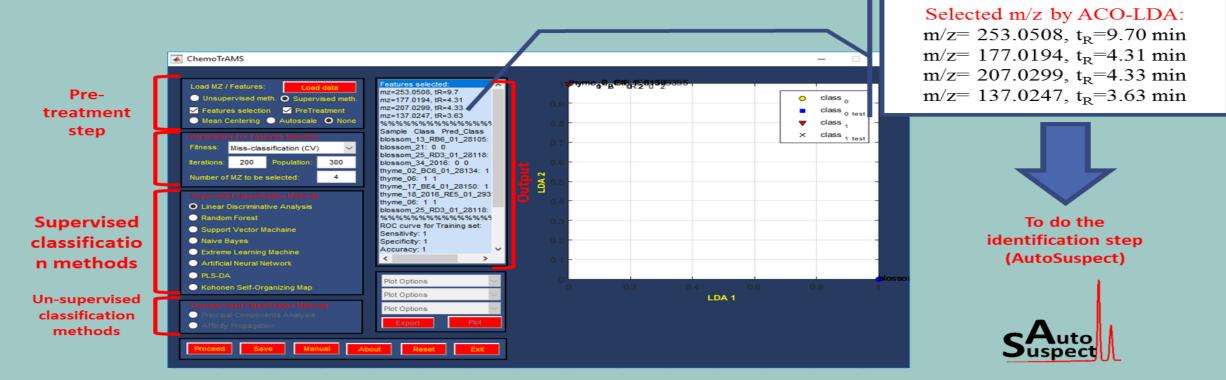
(† (†)



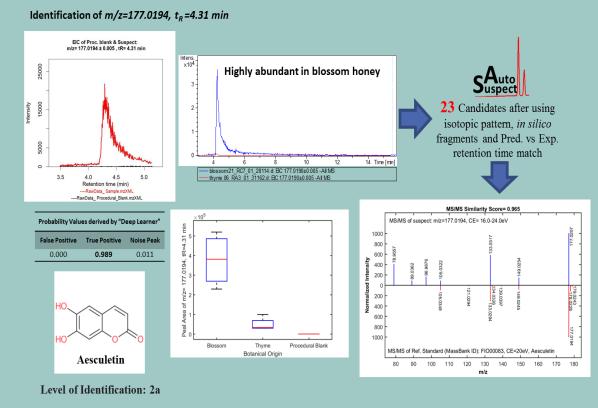
- UHPLC-ESI-MS/MS: (QTOF-MS)(Bruker MaXis Impact)
- Column: Acclaim C18 (Dionex-Thermo Scientific)
- RP chromatographic system
- Elution gradient program
- ESI mode: negative
- Full scan MS and bbCID mode in a single run



Detection of markers in honey by ChemoTrAMS v2.0 Ant Colony Optimization Linear Discriminative Analysis (ACO-LDA)



Identification 2



Identification of 137.0247, t_R=3.63 min



Statistical Analysis

-8 -6 -4 -2 0 2 4 6 8

T thyme 01_BC5_ thyme 11_BD6_01 hyme 03_BC7_01_2

PC1

ssom 04 RA5 01 2809

ossom 17_RC4_01_2811; om 07_RA8_01_28099

m 25_RD3_01_28118

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Variable Importanc

4.71min: 302.17290m/:

nin : 286.08414 m

2min: 152.04760m

TO 100 200 300 400

RT [min]



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	m/z	M meas.	Ions	RT [I	Molecular For	Annotations 🔻	AQ	Boxplot	Flags	MS/MS	Name	ΔRT	∆m/z [mDa]	∆m/z [ppm]	mSigma	MS/MS score	blossom 01_201	5 blossom 02_2016.	blossom 03_RA4	blossom 04_RA5	blosson 🔺
37	193.0	194.05782	+ - •	3.31		C10H10O4	AL SF		•			Ferulic acid	0.31	-0.408	-2.115	00	0.0					
38	137.0	138.03203	+ u	3.63	\checkmark	C7H6O3	AL SF		ĥ II- +			salicylic acid	-0.07	0.356	2.595	3.9	0.0					
39	137.0	138.03204	+ _	3.87		C7H6O3	AL SF		F			salicylic acid	0.17	0.347	2.530	9.1	0.0					
40	151.0	152.04759	+ u	4.45	\checkmark	C ₈ H ₈ O ₃	AL SF		-			Vanillin	-0.35	0.505	3.341	00	0.0					
41	151.0	152.04756	+ <u>-</u>	4.74		C ₈ H ₈ O ₃	AL SF		-			Vanillin	-0.06	0.161	1.068	8	0.0					
42	303.0	304.05804	+ a	4.84	\checkmark	C ₁₅ H ₁₂ O ₇	AL SF	H	p			Taxifolin	-0.06	-0.206	-0.681	00	0.0					
43	269.0	270.05255	+ a	10.11		C15H10O5	AL SF	H	•		du	Galangin	0.11	-0.066	-0.245	00	0.0					
44	287.0	288.06329	+ a	6.36	\checkmark	C ₁₅ H ₁₂ O ₆	AL SF	H	•			Eriodictyol	-0.04	-0.801	-2.792	80	0.0					
45	301.0	302.07890	+ a	7.43		C ₁₆ H ₁₄ O ₆	AL SF	H	• •			Hesperitin	-0.07	-0.684	-2.271	00	0.0					V
46	269.0	270.05273	+ a	8.18	\checkmark	C15H10O5	AL SF	#			վեր	Apigenin	-0.02	-0.065	-0.242	19.3	0.0					
•									L	-											3	4

Bucket Annotation

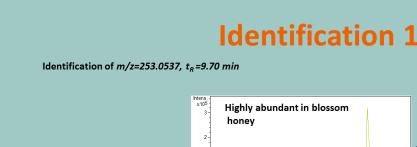
nal	yte List for annotation:	Analyte list for metaboscape annotation											
#	Name	Molecular For	m/z	RT	Main Positi	Main Nega							
1	3,4- dihydroxybenzoic a	C7H6O4	154.0	1.30	[M+H]+	[M-H]1-							
2	2,5-dihydroxybenzoic a	C7H6O4	154.0	2.30	[M+H]+	[M-H]1-	Ξ						
3	4-hydroxybenzoic acid	C7H6O3	138.0	1.40	[M+H]+	[M-H]1-							
4	Apigenin	C15H10O5	270.0	8.20	[M+H]+	[M-H]1-							
5	Caffeic acid	C ₉ H ₈ O ₄	180.0	1.90	[M+H]+	[M-H]1-							
6	Cinnamic acid	C ₉ H ₈ O ₂	148.0	4.50	[M+H]+	[M-H]1-							
7	Epicatechin	C ₁₅ H ₁₄ O ₆	290.0	4.40	[M+H]+	[M-H]1-							
В	Ethyl vanillin	C ₉ H ₁₀ O ₃	166.0	5.60	[M+H]+	[M-H]1-							
9	Ferulic acid	C10H10O4	194.0	3.00	[M+H]+	[M-H]1-							
10	Gallic acid	C7H6O5	170.0	1.30	[M+H]+	[M-H]1-							
11	Hydroxytyrosol	C ₈ H ₁₀ O ₃	154.0	3.50	[M+H]+	[M-H]1-							
12	Luteolin	C15H10O6	286.0	7.50	[M+H1+	[M-H11-	Ŧ						
		Tolerances and Sco	ring										
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_		Retention time:			0.4 [1]	j minutes							
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						_							
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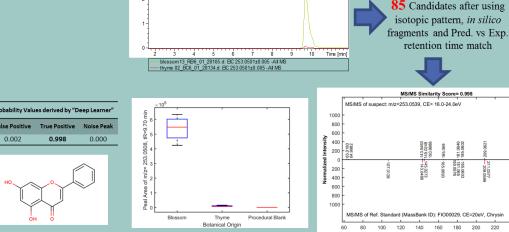
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0/C	0.0	- 1.2	S/	C 0.0	- 0.8	Br/C	0.0	- 0.8	
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Annotat				•					
		mula on:	Ocomplete	Bucket Table					
			Selected B	uckets Only					
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9 buckets seem to be the most influential for the discrimination of Blossom and Thyme honey

5 important buckets are unknown compounds

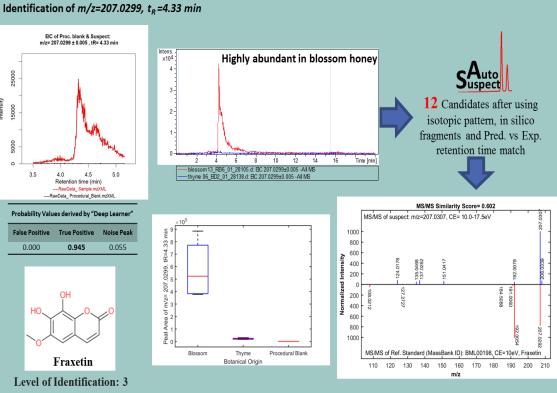
-SE6 0E0 SE6 PC1 🔍





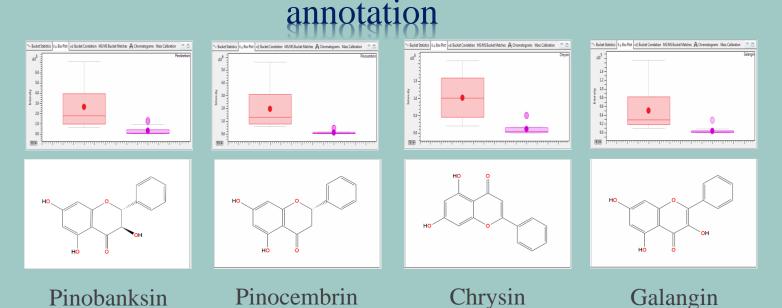
Chrysin Level of Identification: 1

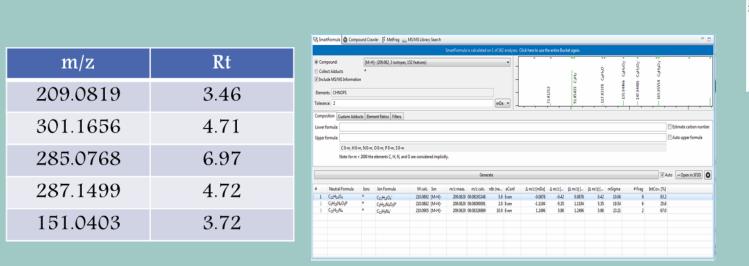
Identification 3

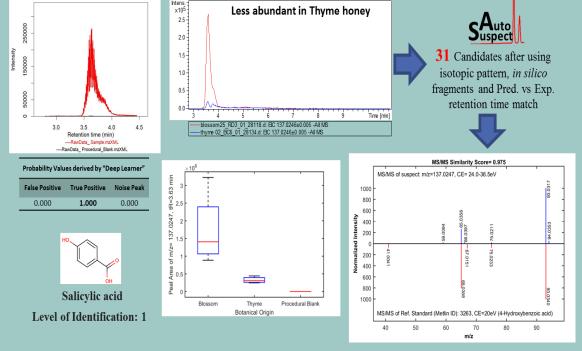


SAuto Suspect

4 buckets are already known from analyte list









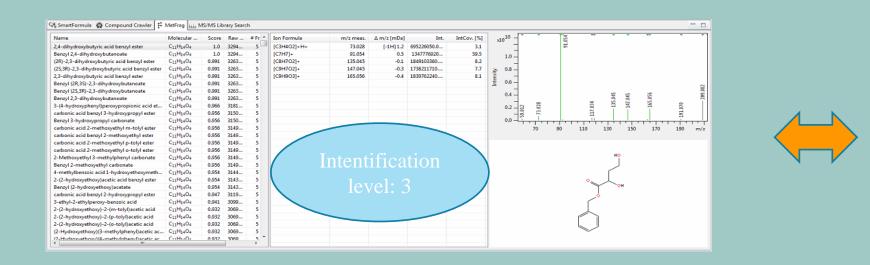
- ✓ Authenticity markers were identified using metaboscape 3.0 for the discrimination of blossom and thyme honey samples.
- ✓ AutoSuspect workflow was successfully implemented for the discrimination of blossom and thyme honeys. New biomarkers were identified.

✓ Chrysin is common in both workflows.

✓ Higher level of identification was reached in metaboscape buckets using AutoSuspect.

Literature

- 1. M. Ciulu, N. Spano, M. Pilo, G. Sanna, Molecules (2016) 21, 451.
- R. Aalizadeh, E. L. Schymanski and N. S. Thomaidis, AutoSuspect: an R package to Perform Automatic Suspect Screening based on Regulatory Databases, 15th International Conference on Environmental Science and Technology, CEST2017.



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