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Laboratorul SNIF-RMN

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Chisinau, Septembrie 2013

RMN – Rezonanta magnetica nucleara

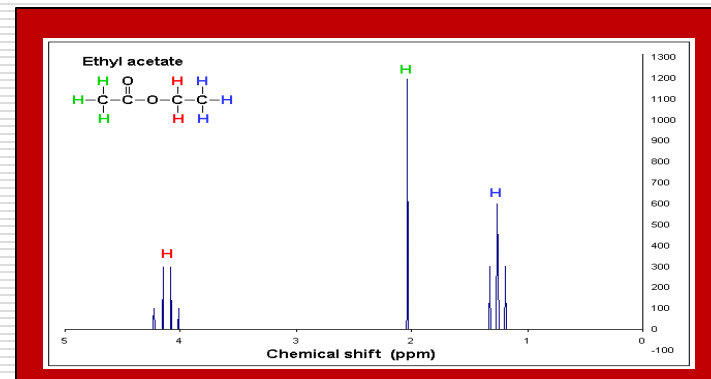
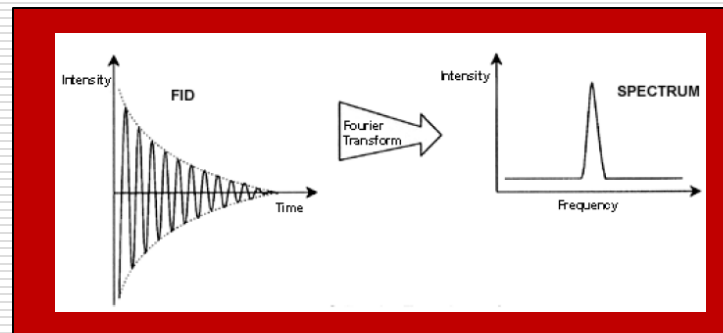
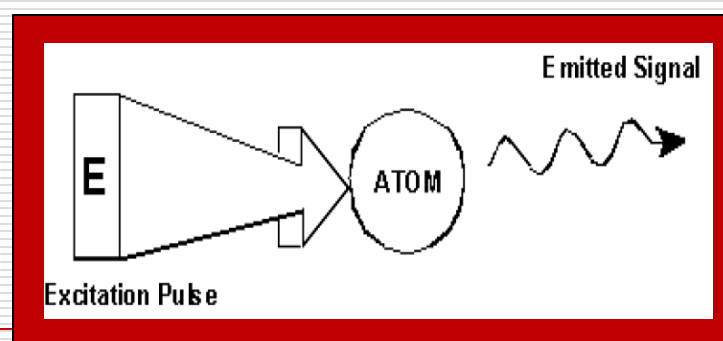
Tehnica RMN este folosita in:

- determinarea structurii moleculelor, in special a compusilor organici si in
- determinarea componentilor din amestecurile complexe.

O analiza RMN se realizeaza in 3 etape:

- 1) pozitionarea probei in camp magnetic;
- 2) excitarea nucleului cu un puls de radiofrecventa (RF) – **frecventa de rezonanta** a nucleului atomului studiat (1H, 13C, D etc.) peste care se suprapune efectul atomilor/gruparilor invecinati – **deplasarea** frecventei;

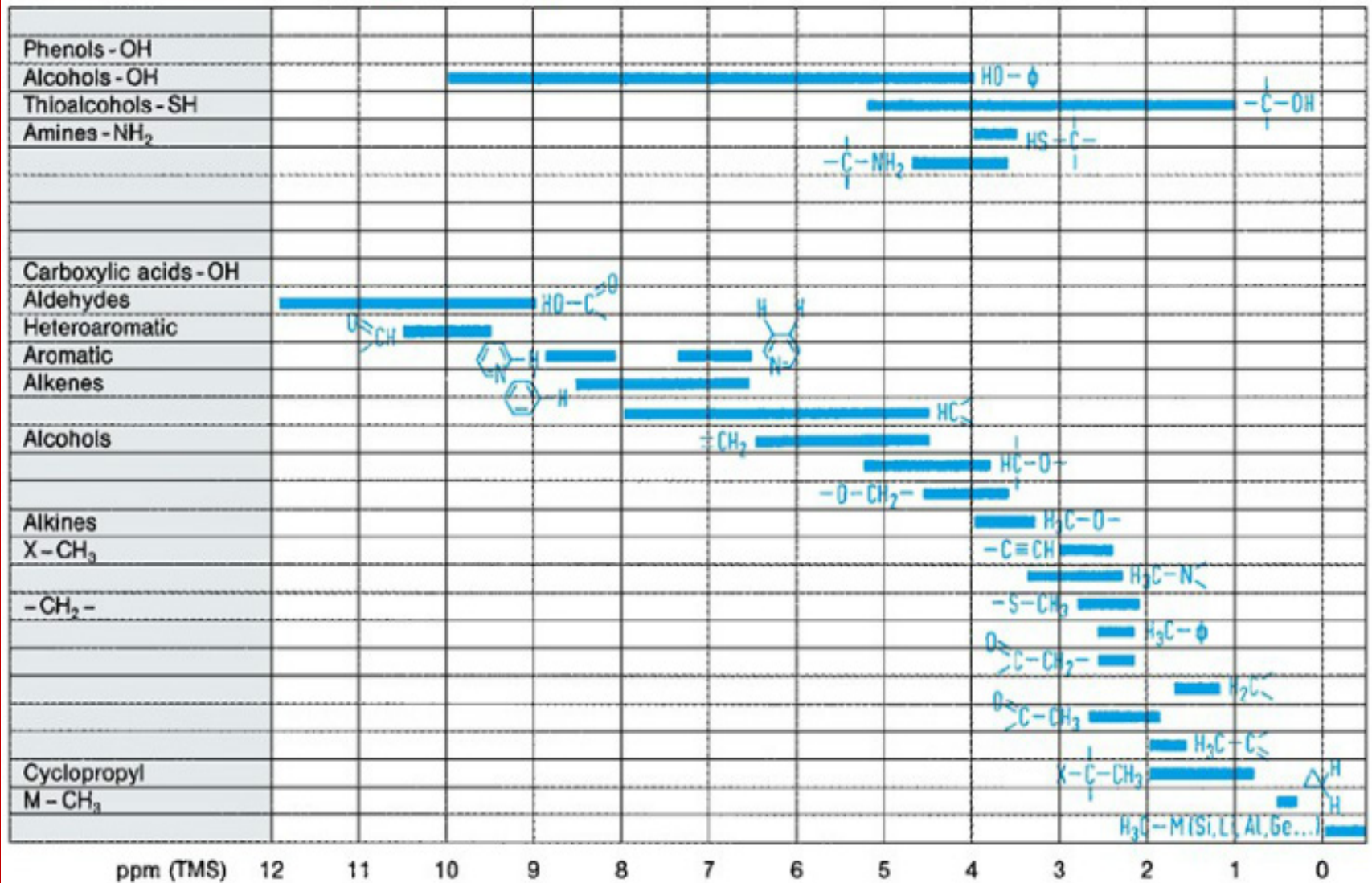
- 3) masurarea frecventei semnalului emis de proba – spectrul RMN (in unitati **ppm** de **deplasare chimica**) – informatii despre gruparile chimice din molecula si structura acesteia. Aria semnalului este direct proportionala cu numarul atomilor echivalenti ce contribuie la acesta.



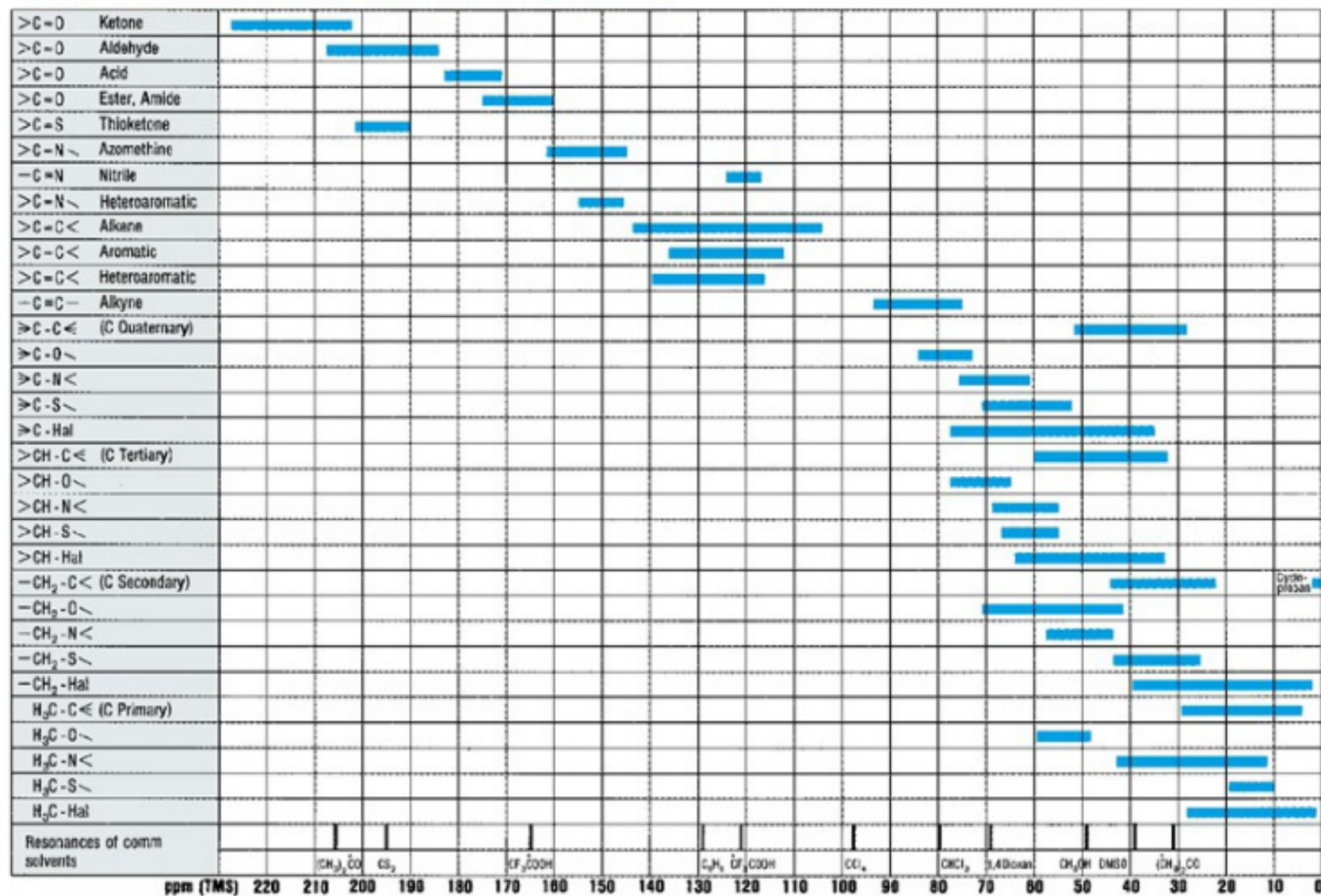
Izotopi utilizati in RMN

Izotop	Abundenta naturala (%at.)	Frecventa de rezonanta (pt 9.4T sau 400MHz)	Fiola - Volum proba - Concentratie proba
1H - Protii	99,9885	400,13	5mm - 0,5ml - 0,1%
2H - Deuteriu	0,0115	61,42	10mm - 5ml - 0,65%
13C - Carbon	1,07	100,61	5mm - 0,5ml - 10%
15N- Azot	0,364	40,56	5mm - 0,5ml - 3%
31P - Fosfor	100	161,97	5mm - 0,5ml - 0,1%
etc.

¹H Chemical Shifts in Organic Compounds



¹³C Chemical Shifts in Organic Compounds*



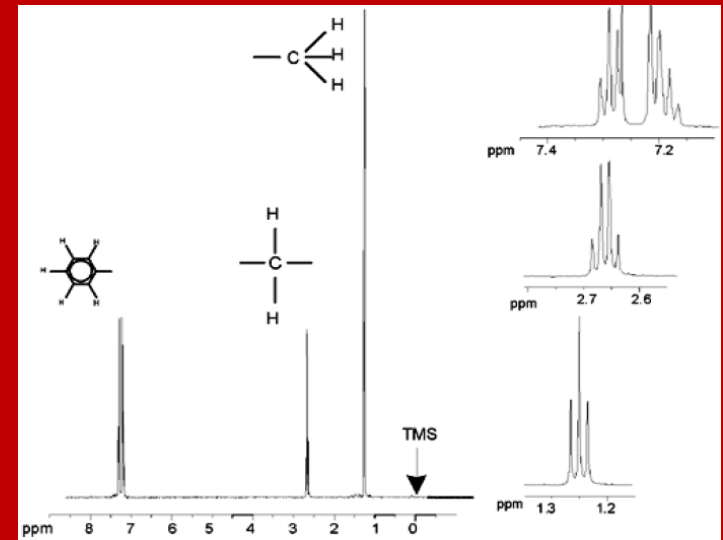
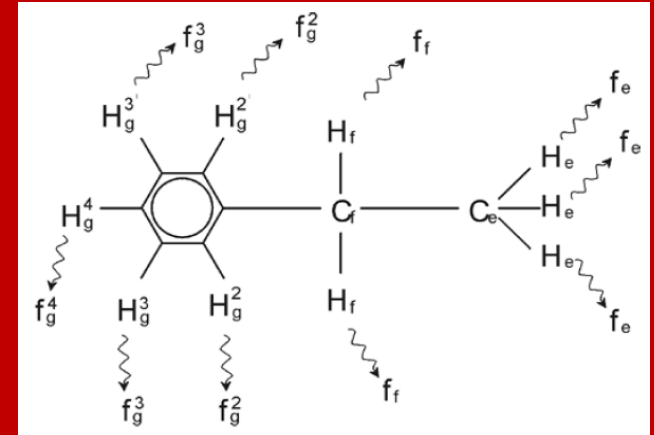
Cuplajul spin-spin

Splitarea semnalului RMN in dublet, triplet, cvartet, dublet de dublete, multiplet etc., se datoreaza interactiunii magnetice cu atomii invecinati.

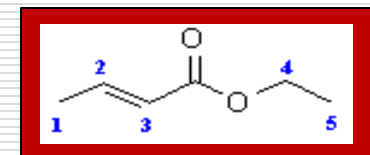
Multiplicitatea semnalului = nr. atomi echivalenti invecinati + 1

Multiplicitatea poate fi cumulativa (dublet de dublete, multiplet).

Atomii echivalenti nu produc splitare si nici deplasare chimica unul fata de celalalt.

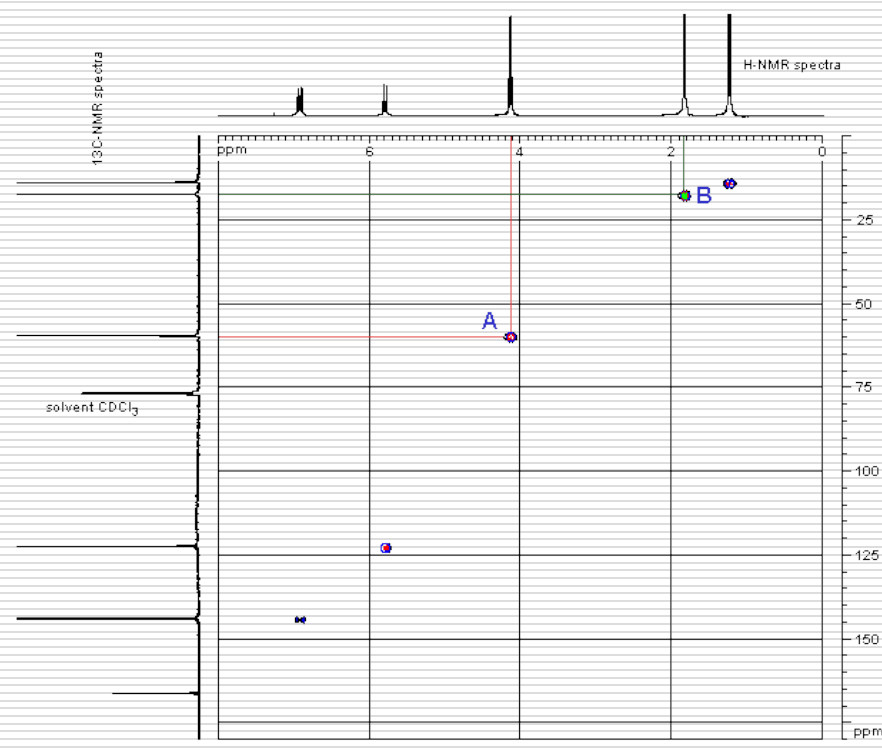
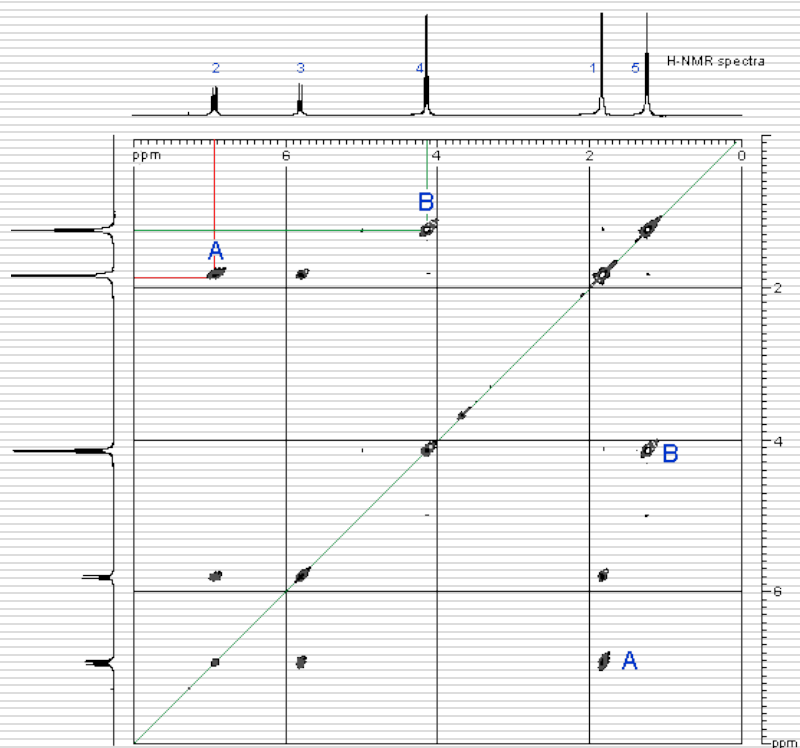


Spectroscopie 2D - RMN



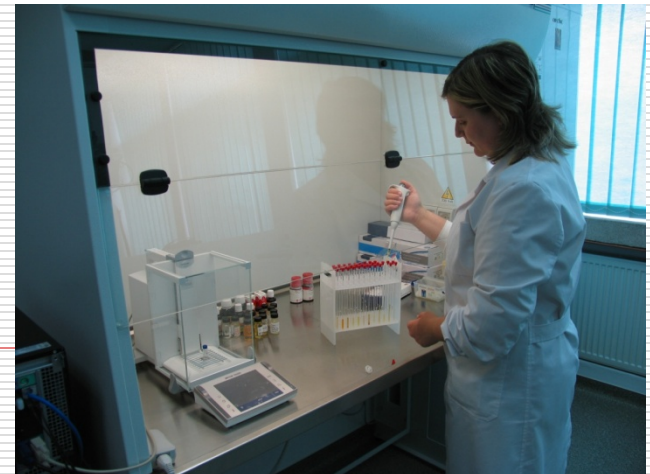
Utilizata in determinarea structurii moleculelor complexe.

Spectroscopie homonucleara ($^1\text{H} - ^1\text{H}$, $^{13}\text{C} - ^{13}\text{C}$) si heteronucleara ($^1\text{H} - ^{13}\text{C}$).



Spectrometrul RMN Bruker 400MHz

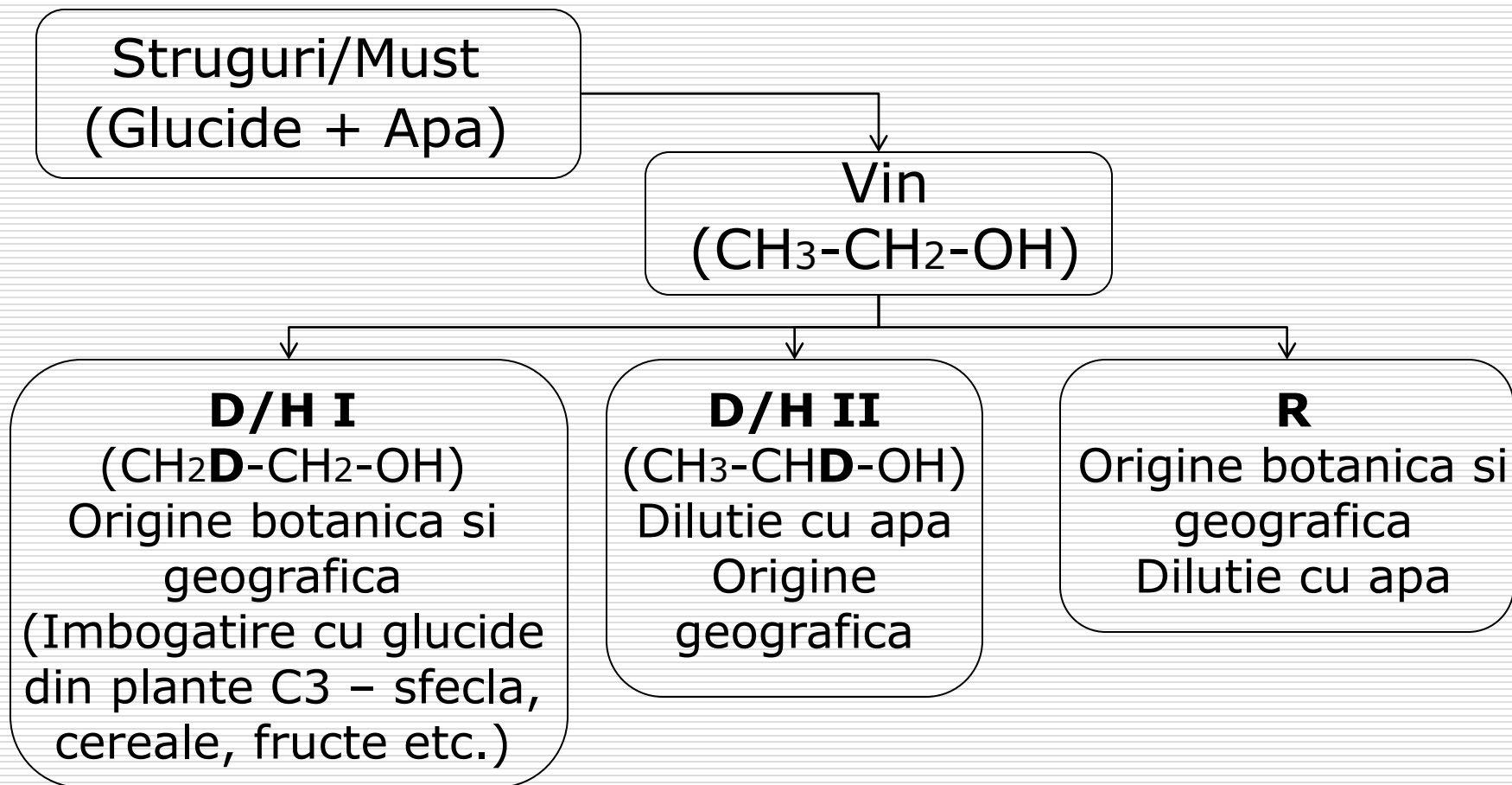
- Caracteristici tehnice:*
 - Spectrometru Ascend400;
 - Consola electronica AvanceIII 400;
 - Autosampler SampleXpress – 60 pozitii;
 - Sonda SNIF (D) si sonda BBO (1H, 13C etc.);
 - Soft de operare – TopSpin 3.0;
 - Magnet supraconductor;
 - Camp magnetic - 9.4T;
 - Frecventa protonului – 400MHz.
-



Informatii generale

- Laboratorul a fost infiintat in februarie 2012;
 - Aplicatia principala - **Autentificarea bauturilor alcoolice, a sucurilor din fructe si/sau legume si a mierii prin tehnica SNIF-RMN** (Site-specific Natural Isotope Fractionation – Nuclear Magnetic Resonance) - rezonanta magnetica nucleara pentru determinarea fractionarii izotopice naturale a deuteriului la gruparile specifice etanolului – metoda OIV standardizata (Organizatia Internationala a Viei si Vinului) in Compendiul metodelor internationale de analiza a vinului si mustului si cel al bauturilor spirtoase de origine viticola;
 - Alte aplicatii – profiling RMN alimente, determinari structurale etc.
-

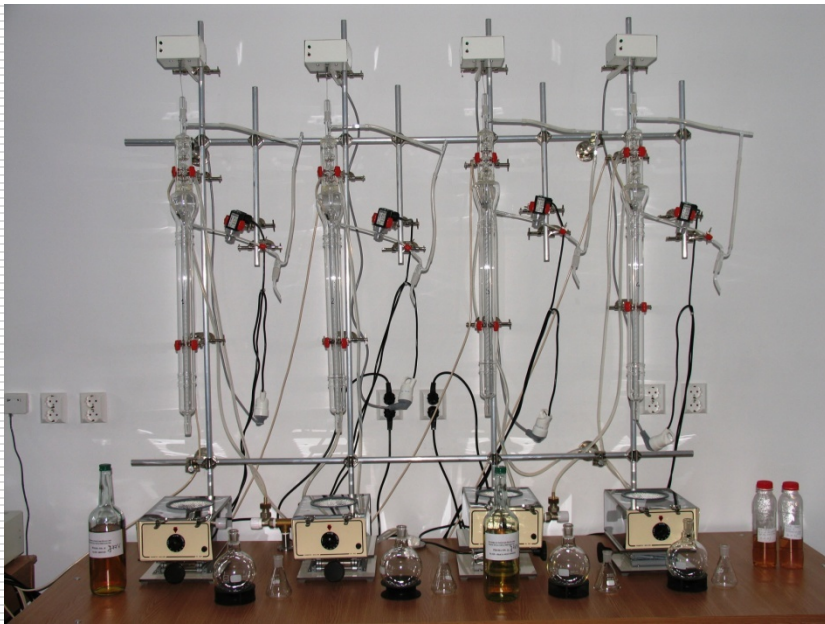
Autentificarea bauturilor prin SNIF-RMN



Autentificarea bauturilor prin SNIF-RMN

□ *Etape:*

- Proba – alcool de 85 – 93%*m* extras din bautura alcoolica sau suc fermentat;
 - **PrepSamp**: soft de introducere a cantitatilor de proba si reactivi luate in lucru;
 - **TopSpin 3.0**: soft de obtinere a spectrelor RMN ale probelor de alcool (200 scanari, 10 spectre, durata 4h, FID 16384, SW 20ppm, AQ 6.68s, lock 19F, frecventa D - 61.42MHz, solvent C6F6).
 - **EuroSpec**: soft de calcul al rezultatelor (D/H I, D/H II si R) si emitere a raportului de analiza.
 - Compararea rezultatelor cu baza de date.
-



ETHANOL

SAMPLE NUMBER:13050286

Spectrometer Filename:

Nb Spectra : 10

Preparation Parameters:

Product Weight : 2.60070g

Purity: 0.9208

Reference Weight: 1.25440g

Reference name:05182C(141.90ppm)

Treatment:

Type of processing :EthanolReprocessing

Results:

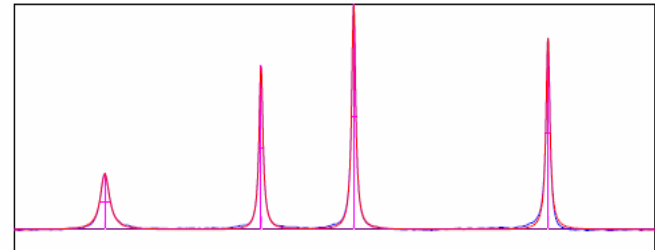
Spectrum	(D/H)1	Intensity	(D/H)2	Area	(D/H)1	(D/H)2
1	100.44	127.95	100.82	126.53		
2	100.60	128.59	101.28	128.34		
3	100.37	127.53	100.53	126.95		
4	100.27	127.87	101.18	127.08		
5	100.24	128.15	99.44	127.08		
6	100.30	127.38	99.84	127.09		
7	100.44	127.78	99.92	126.97		
8	100.12	127.44	99.90	126.94		
9	100.37	126.83	100.34	126.13		
10	100.18	128.18	100.11	126.61		
Mean	100.33	127.77	100.34	126.97		
SD	0.14	0.50	0.61	0.57		

Ratio R (Intensity)

Mean : 2.547 (0.010)

Largeurs de raies

	CH3	TMU
1	2.469	2.460
2	2.459	2.442
3	2.463	2.459
4	2.471	2.449
5	2.440	2.460
6	2.452	2.463
7	2.448	2.460
8	2.463	2.468
9	2.457	2.457
10	2.443	2.445



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Z:\snfmm\13050286\10\fid

5/17/2013 7:51:03 AM

Sample Edit Print Communication Quit

Run Session

Session Number: 130910B Molecule: ETHANOL Sample: 1 / 6

Date: 10/09/2013 Name: Préparation standard Add Lst (F12)

Operator: A12 Minimum Quantity: 3.20 ml Check Order (Alt+F12)

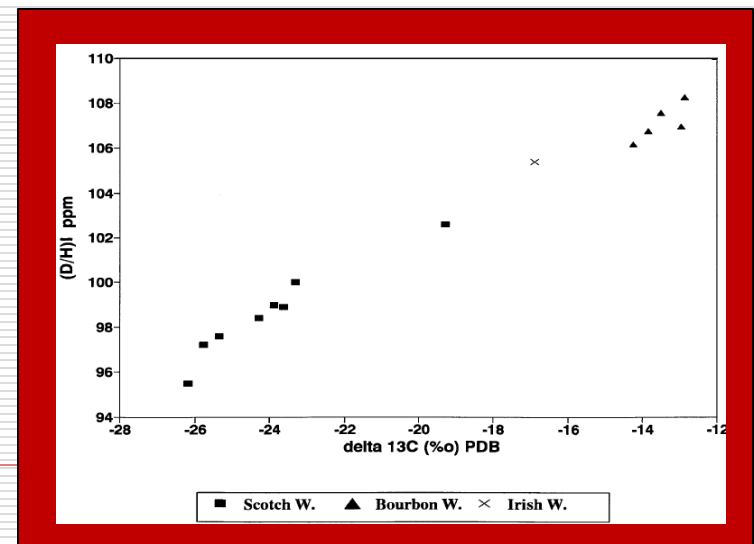
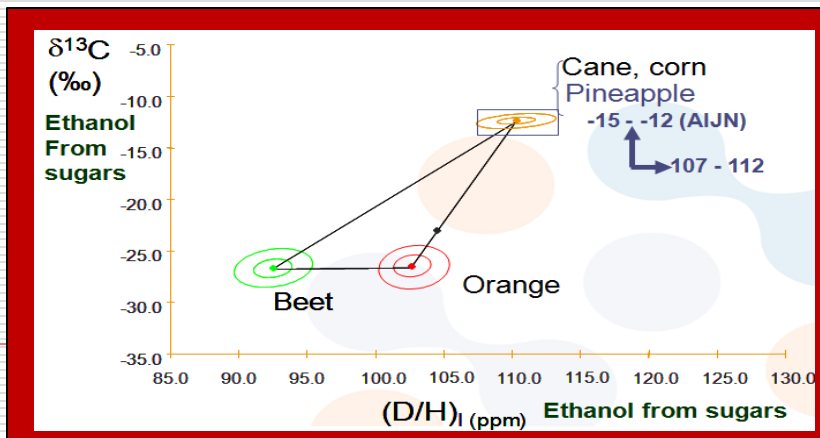
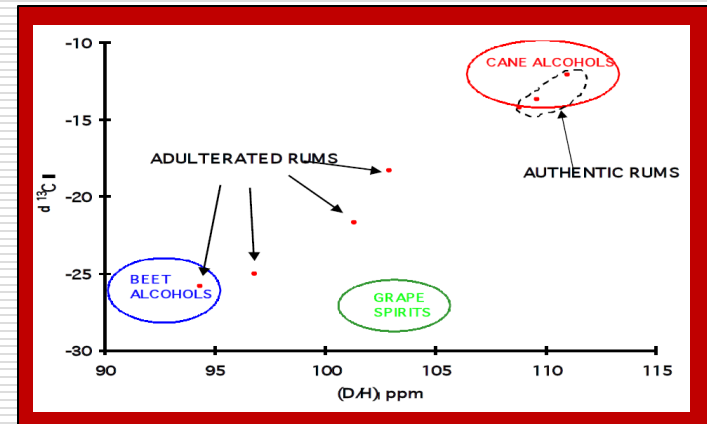
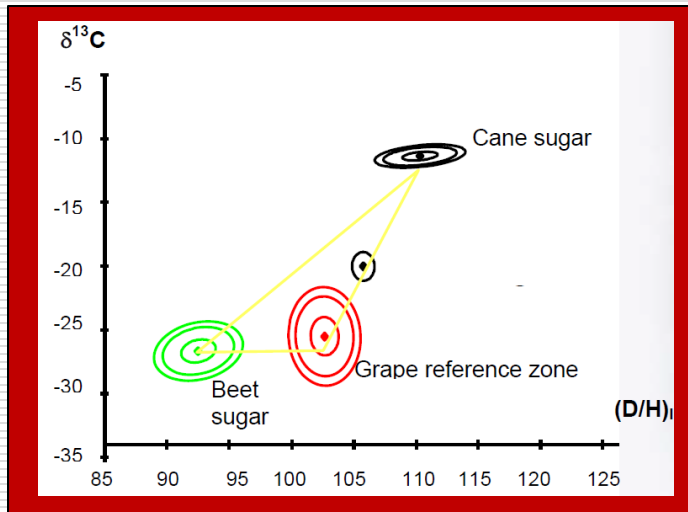
Sample: Prev (F2) 13090493 Next (F1)

Standard ref.: 05182H Td: 0.9202 w/w Total weight calc.: 28.41790 g Import all tm values (F7)

Type	Name	Quantity	Unit	Qty. Calc.	Cont/Weight	Total Weight	Valid
Empty flask		0.0000		0.0000	24.29480	24.29490	✓
Standard	TMU	1.3000	ml	1.3023	1.26150	25.55640	✓
Sample		3.2000	ml	3.3430	2.63830	28.19470	✓
Lock	C6F6+CF3CO	0.1500	ml	0.1385	0.22330	28.41800	✓

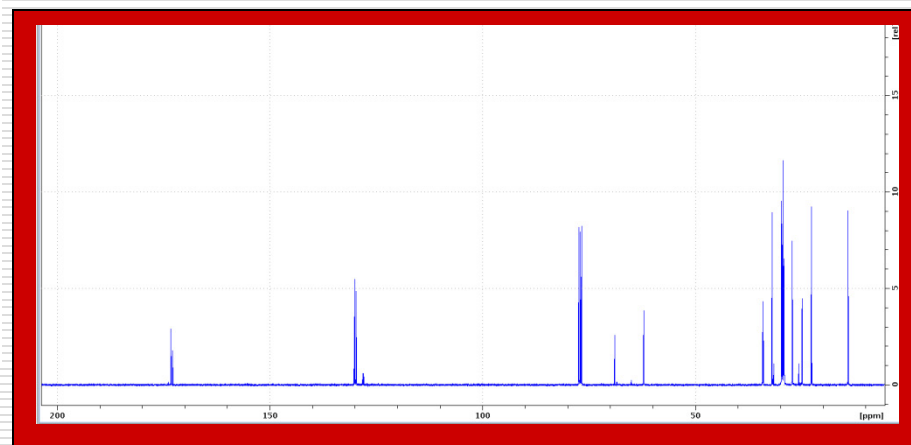
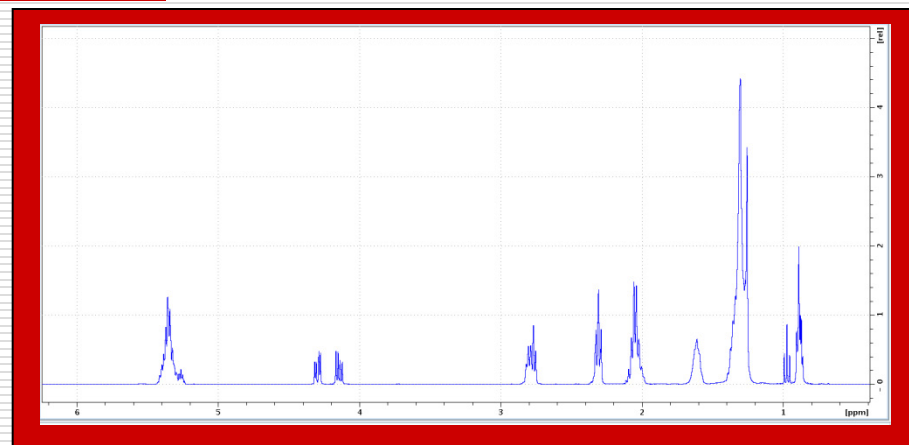
Save Session Design Session Report Design Label Report

Interpretarea rezultatelor analizei SNIF-RMN – Baze de date



Profil RMN - Uleiuri

Chemical shift δ (ppm)	Proton	Compound
0.87	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_3$	All acids except linolenyl
1.02	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$	Linolenyl
1.30	$(\text{CH}_2)_n$	All acyl chains
1.62	$-\text{CH}_2-\text{CH}_2-\text{COOH}$	All acyl chains
2.03	$-\text{CH}_2-\text{CH}=\text{CH}-$	All unsaturated fatty acids
2.32	$-\text{CH}_2-\text{COOH}$	All acyl chains
2.77	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$	Linoleyl and linolenyl
4.22	$-\text{CH}_2-\text{OCO}-\text{R}$	Glycerol (triacylglycerols)
5.26	$\text{CH}-\text{OCO}-\text{R}$	Glycerol (triacylglycerols)
5.37	$-\text{CH}=\text{CH}-$	All unsaturated fatty acids



Componente cuantificate: **total acizi saturati, indicele de iod** (gradul global de nesaturare), **acidul oleic, acidul linolenic, acidul linoleic.**

Profil RMN - Miere

Componente

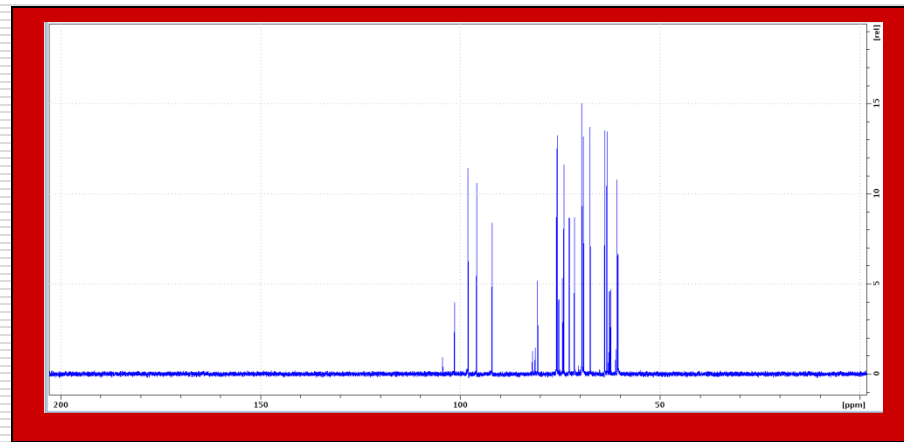
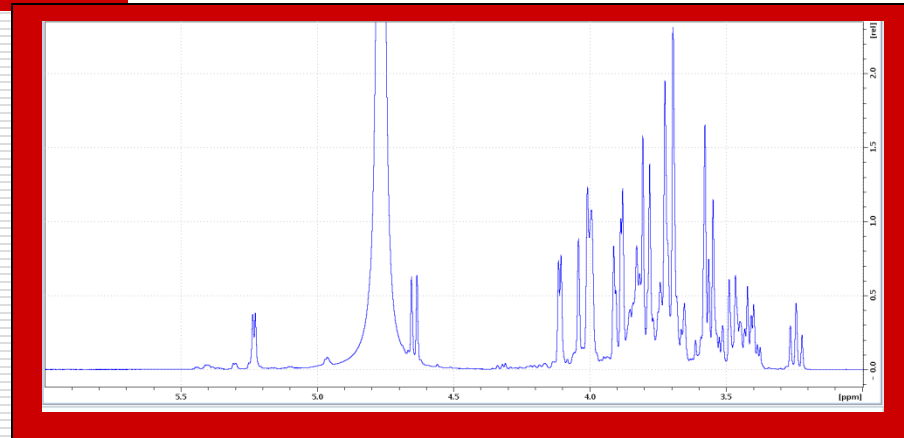
cuantificate/identificate:

- **Glucide** (raportul F/G, total glucide, glucoza, fructoza, zaharoza, turanoza etc.);

- **Acizi organici** (acid lactic t-1,35ppm, acid acetic, acid formic s-8,45ppm etc.);

- **Aminoacizi** (alanina d-1,46ppm, izoleucina m 1,95-2.03ppm, acid glutamic/glutamat m-2,34ppm, glutamina m-2,45ppm, fenilalanina, tirozina, prolina etc.);

- **Alcooli** (etanol t-1,15ppm).

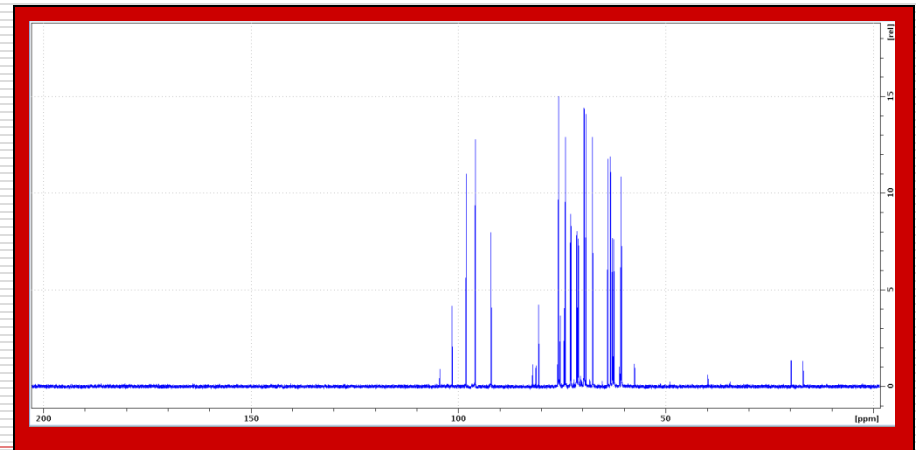
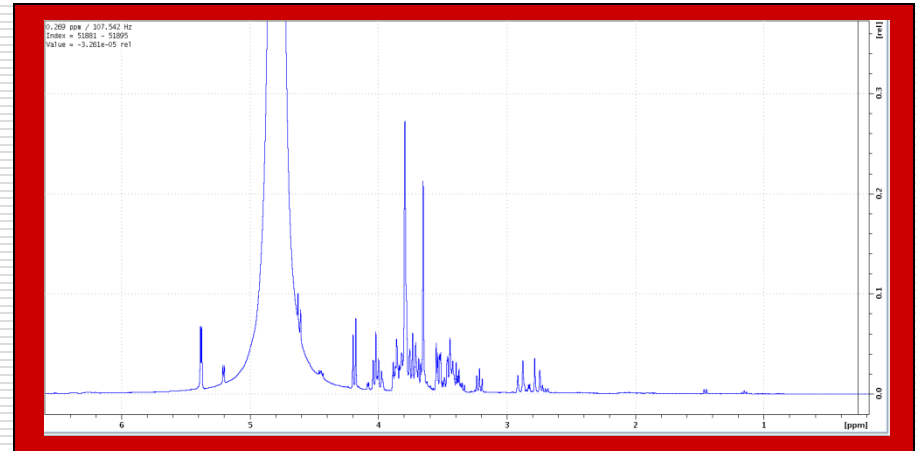


Profil RMN – Fructe/Sucuri de fructe

Componente

cuantificate/identificate:

- **Glucide** (glucoza , fructoza, zaharoza, turanoza etc.);
- **Acizi organici** (acid citric, acid maleic, acid ascorbic, acid tartric, acid succinic etc.);
- **Aminoacizi**;
- **Alcooli** (etanol, metanol, glicerina etc.).

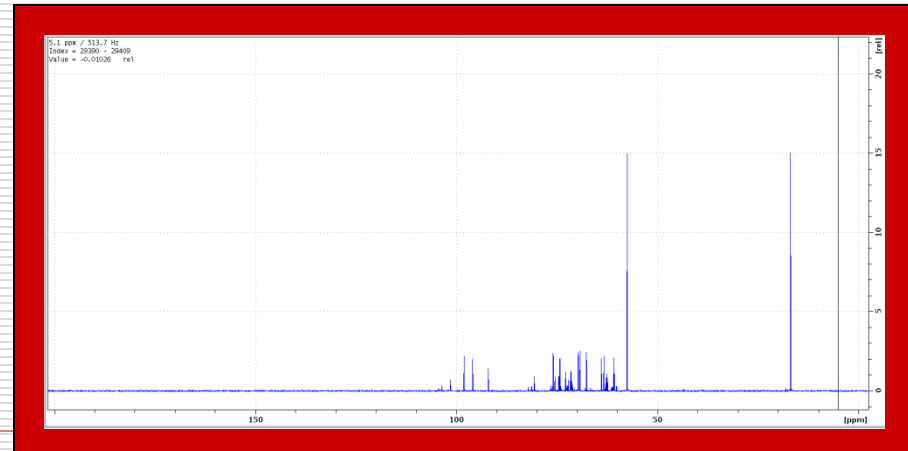
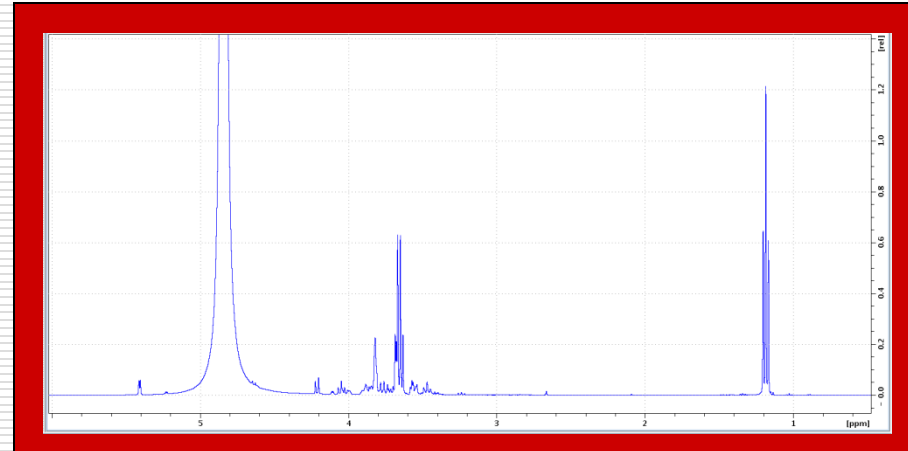


Profil RMN - Vin

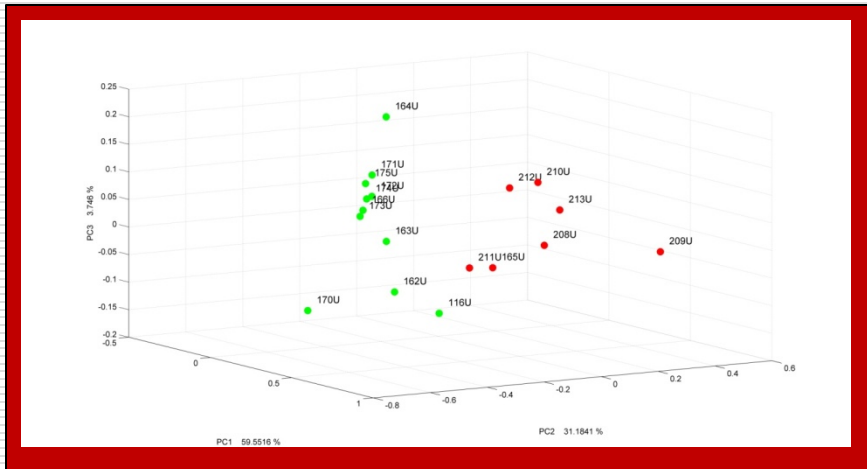
Componente

cuantificate/identificate:

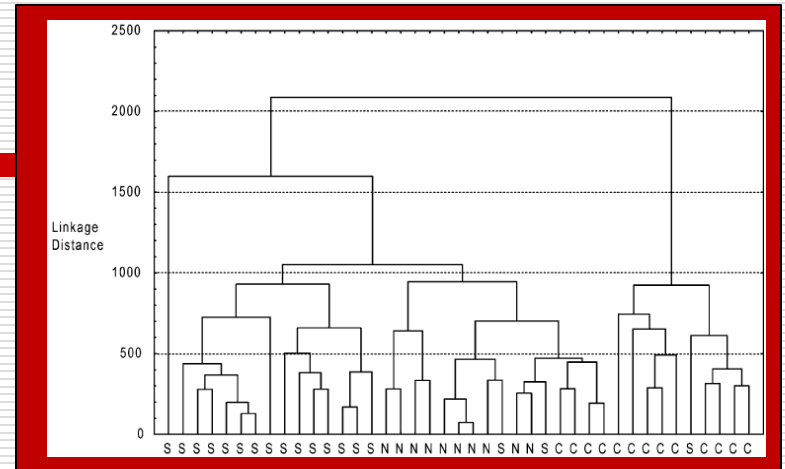
- **Glucide** (glucoza , fructoza, zaharoza, turanoza etc.);
- **Acizi organici** (acid citric, acid maleic, acid ascorbic, acid tartric, acid succinic etc.);
- **Aminoacizi**;
- **Alcooli** (etanol, metanol, glicerina, 2,3-butandiol etc.).



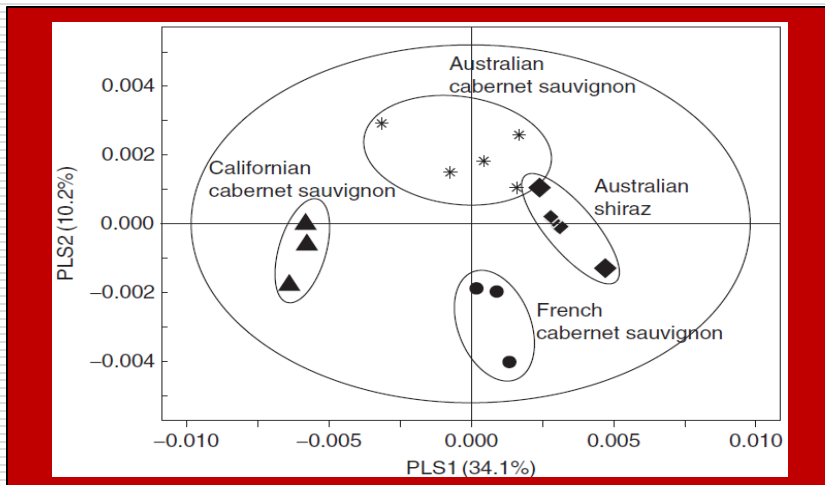
Analiza datelor - Chemometrie



PCA (Principal Component Analysis) – probe de ulei de masline si floarea soarelui



Dendrograma 1H-RMN – vinuri rosii din nordul, sudul si centrul Italiei (Brescia si colab., 2002)



PLS-DA (Partial Least Squares Discriminant Analysis) (Son si colab., 2008)

Concluzii

- ❑ Tehnica SNIF-RMN permite determinarea originii botanice si certificarea originii geografice a bauturilor alcoolice si nealcoolice.
 - ❑ Tehnica RMN ofera un numar foarte mare de informatii privind compozitia alimentelor intr-un timp relativ scurt si cu preparare minima a probei.
 - ❑ Se pot construi baze de date cu spectrele RMN ale alimentelor autentice, ce pot fi folosite ulterior in autentificarea probelor necunoscute.
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