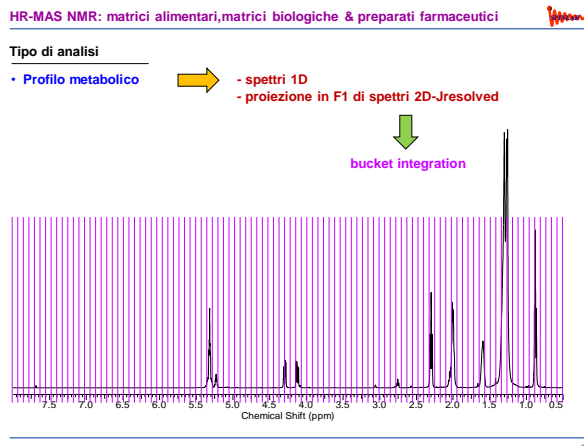
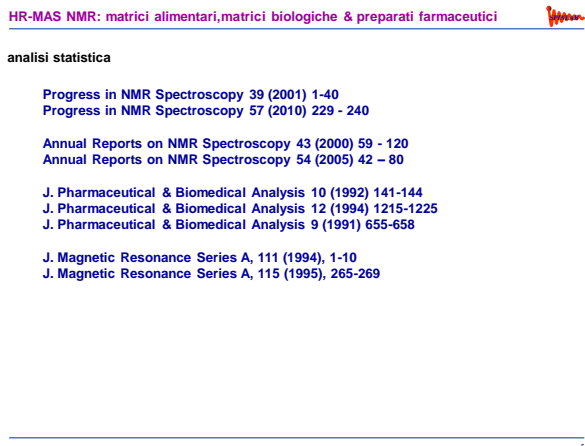
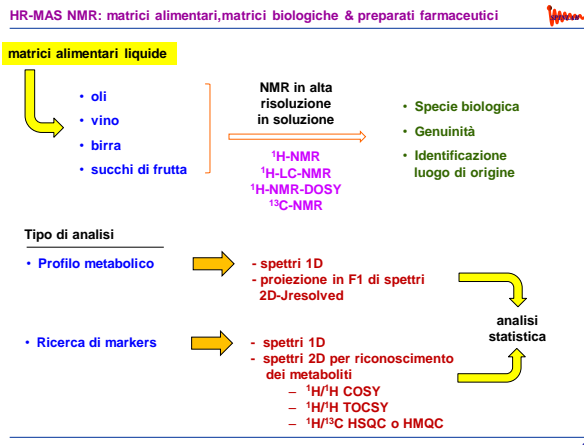
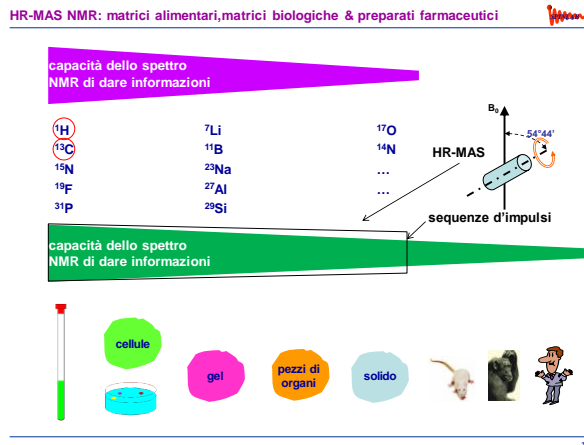


# HR-MAS Workshop

Centro Interdipartimentale Misure  
"Giuseppe Casnati"  
Università di Parma  
31 marzo 2014

## HR-MAS NMR

Matrici alimentari,matrici biologiche e preparati farmaceutici





Spettri NMR HRMAS - Matrici alimentari

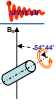
Matrici

- Frutti
- Verdure
- Semi
- Tuberi
- Farine
- Carni
- Formaggi
- Piante medicinali

mozzarella	Food Chemistry 132 (2012) 1620–1627
emmental	J. Agric. Food Chem. 2006, 54, 4148–4154
Hengshanhuangqi and Chuanhuangqi	Yao Xue Xue Bao. 2013 Oct;48(10):1595–601.
saw palmetto products	J Pharm Pharmacol. 2014 Jan 13.
"Curcuma longa"	J Ethnopharmacol. 2014 Jan 10
Tabacco (Autenticità sigarette)	Anal Bioanal Chem. 2013;405(28):9093–100.

- Succhi di frutta

Magn. Reson. Chem. 2011, 49, S121–S125



Spettri NMR HRMAS - Matrici alimentari

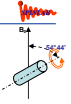
Matrici

- ✓ Frutti
- Verdure
- Semi
- Tuberi
- ✓ Farine
- ✓ Carni
- ✓ Formaggi
- Piante medicinali

fave, mele, kiwi

farine/semole  
carni vari tagli  
parmigiano-reggiano

Caratterizzazione  
luogo di origine  
&  
qualità del prodotto



Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz

Fava liofilizzata e ripresa con D<sub>2</sub>O

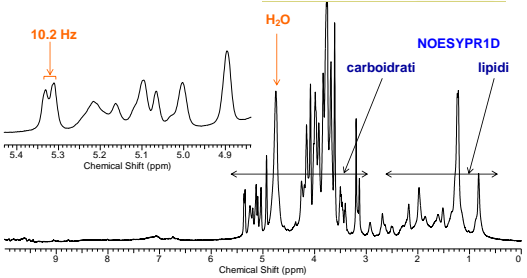
Spinning rate = 4000 g/s;

Rotore Ø = 4 mm; SW = 8400 Hz;

No. data points = 16K;

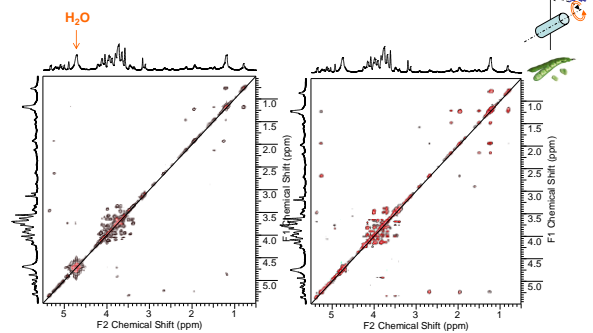
No. transients = 64

Grassi	0.22 g
Carboidrati	12.41 g
Proteine	5.47 g
Fibre	3.70 g
Acqua	80.32 g
Ceneri	1.58 g



Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz

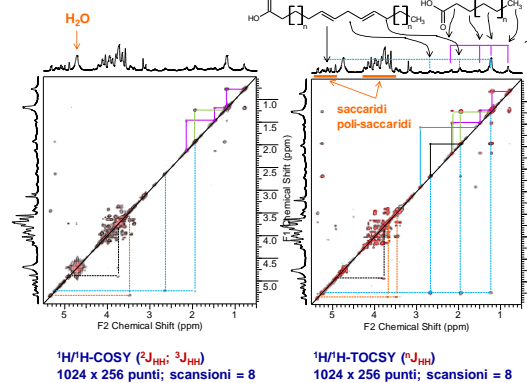


<sup>1</sup>H/<sup>1</sup>H-COSY (<sup>2</sup>J<sub>HH</sub>; <sup>3</sup>J<sub>HH</sub>)  
1024 x 256 punti; scansioni = 8

<sup>1</sup>H/<sup>1</sup>H-TOCSY (<sup>1</sup>J<sub>HH</sub>)  
1024 x 256 punti; scansioni = 8

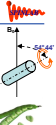
Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz



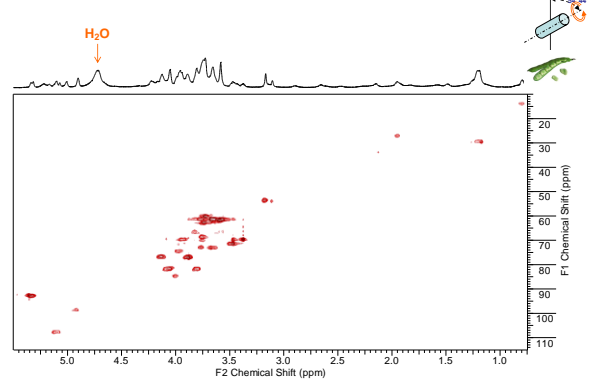
<sup>1</sup>H/<sup>1</sup>H-COSY (<sup>2</sup>J<sub>HH</sub>; <sup>3</sup>J<sub>HH</sub>)  
1024 x 256 punti; scansioni = 8

<sup>1</sup>H/<sup>1</sup>H-TOCSY (<sup>1</sup>J<sub>HH</sub>)  
1024 x 256 punti; scansioni = 8



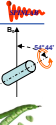
Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz



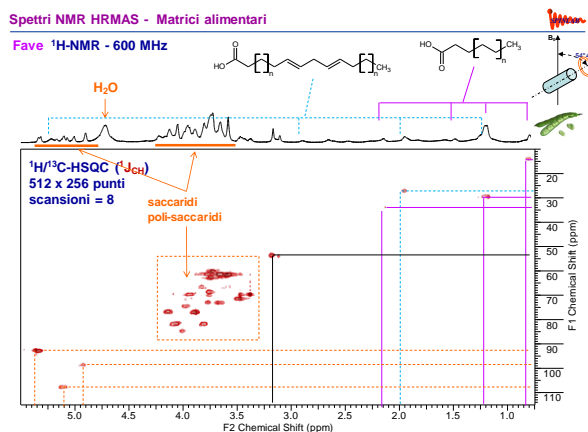
<sup>1</sup>H/<sup>1</sup>H-COSY (<sup>2</sup>J<sub>HH</sub>; <sup>3</sup>J<sub>HH</sub>)  
1024 x 256 punti; scansioni = 8

<sup>1</sup>H/<sup>1</sup>H-TOCSY (<sup>1</sup>J<sub>HH</sub>)  
1024 x 256 punti; scansioni = 8



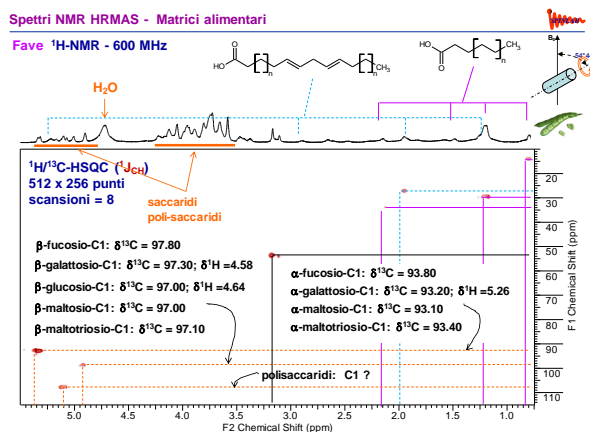
# Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz



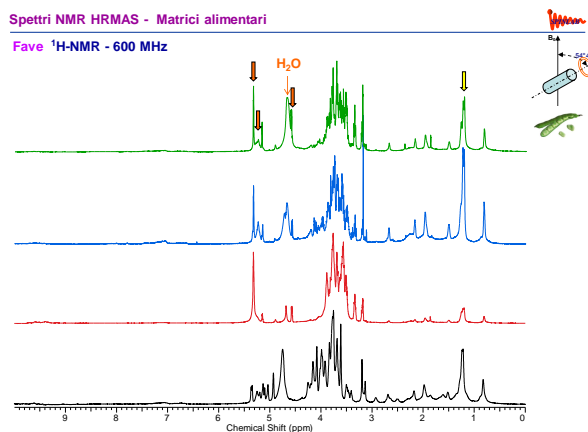
# Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz



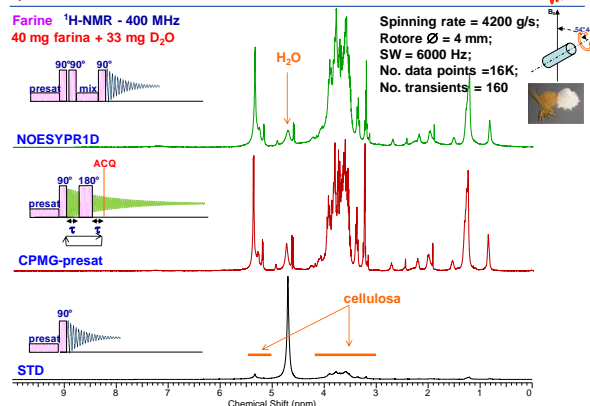
# Spettri NMR HRMAS - Matrici alimentari

Fave <sup>1</sup>H-NMR - 600 MHz



# Spettri NMR HRMAS - Matrici alimentari

Farine <sup>1</sup>H-NMR - 400 MHz

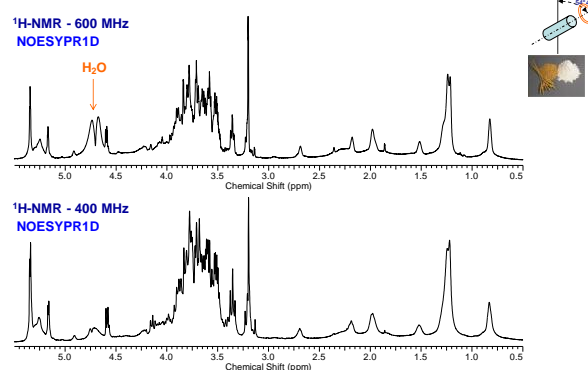


# Spettri NMR HRMAS - Matrici alimentari

Farine

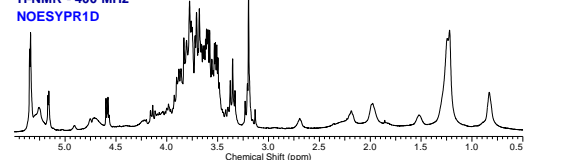
<sup>1</sup>H-NMR - 600 MHz

NOESYPR1D



<sup>1</sup>H-NMR - 400 MHz

NOESYPR1D

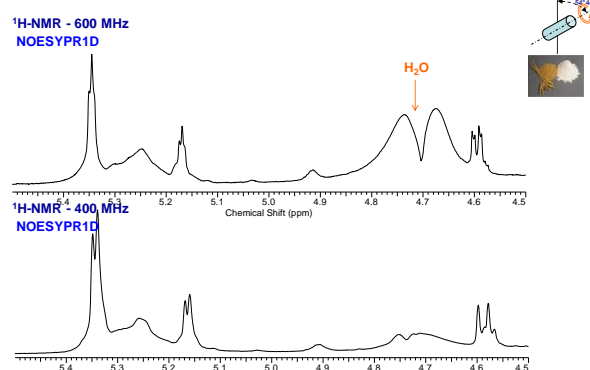


# Spettri NMR HRMAS - Matrici alimentari

Farine

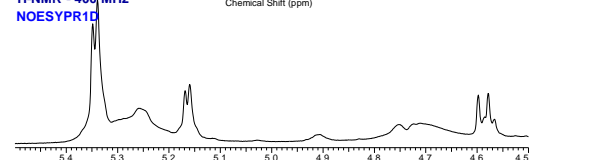
<sup>1</sup>H-NMR - 600 MHz

NOESYPR1D



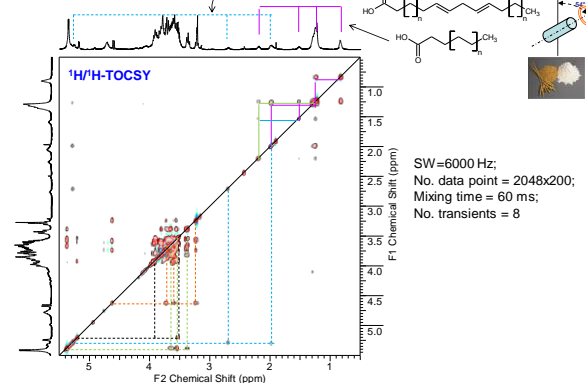
<sup>1</sup>H-NMR - 400 MHz

NOESYPR1D



# Spettri NMR HRMAS - Matrici alimentari

Farine <sup>1</sup>H-NMR - 400 MHz

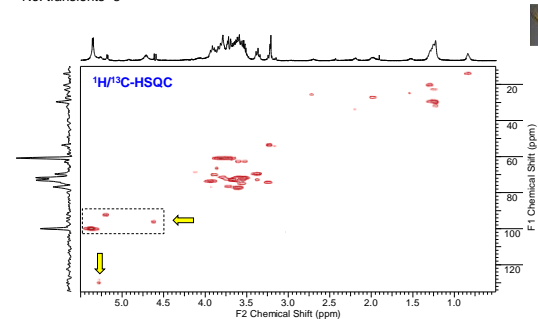


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# Spettri NMR HRMAS - Matrici alimentari

Farine <sup>1</sup>H-NMR - 400 MHz

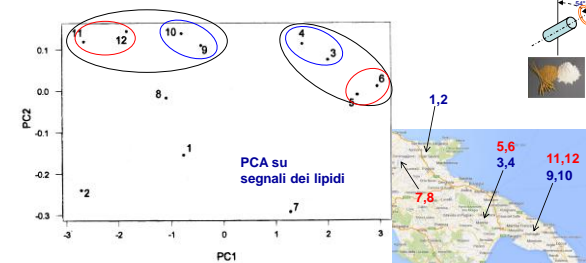
SW=6000 Hz;  
No. data points =512x200;  
No. transients=8



26

# Spettri NMR HRMAS - Matrici alimentari

Farine



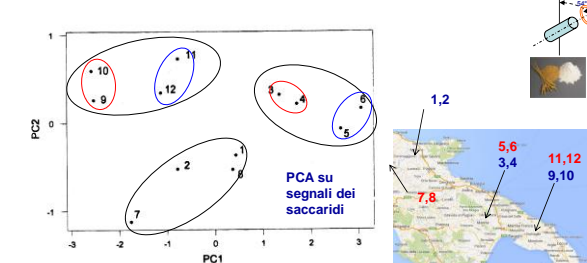
**Figure 7.** Scatter plot of the scores from the first two principal components PC1 and PC2 for durum wheat flour samples using the spectral data obtained from <sup>1</sup>H NOESY sequence in the range 0.7–2.8 ppm: S. Severo Ofanto (1, 2); Matera Fortore (3, 4); Matera Ofanto (5, 6); Montelongo Ofanto (7, 8); Francavilla Fortore (9, 10); Francavilla Ofanto (11, 12). PCA = Principal Component Analysis

S. Ghelli, A. Sacco, M. Spraul, E. Humpfer et al.J. Agric. Food Chem. 46(10), (1998) 4242-4249

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# Spettri NMR HRMAS - Matrici alimentari

Farine



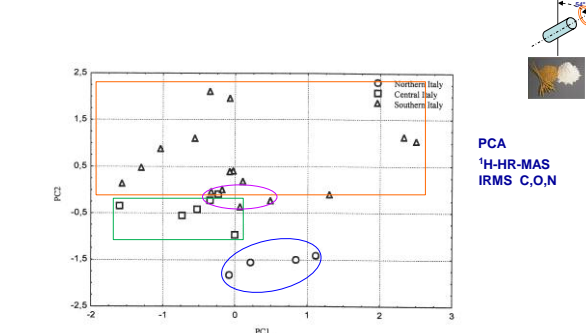
**Figure 8.** Scatter plot of the scores from the first two principal components PC1 and PC2 for durum wheat flour samples using the spectral data obtained from <sup>1</sup>H NOESY sequence in the range 3.0–3.4 ppm: S. Severo Ofanto (1, 2); Matera Fortore (3, 4); Matera Ofanto (5, 6); Montelongo Ofanto (7, 8); Francavilla Fortore (9, 10); Francavilla Ofanto (11, 12).

S. Ghelli, A. Sacco, M. Spraul, E. Humpfer et al.J. Agric. Food Chem. 46(10), (1998) 4242-4249

28

# Spettri NMR HRMAS - Matrici alimentari

Farine



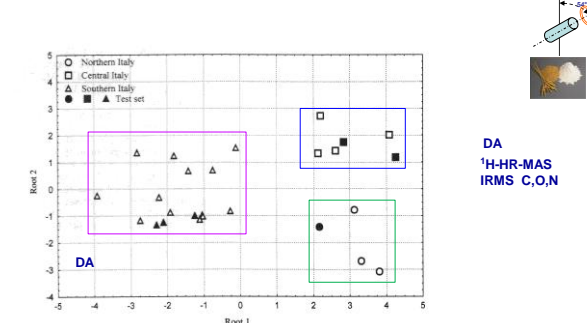
S. Ghelli, A. Sacco et al.J. Cereal Chem. 79(2), (2002) 238-242  
S. Ghelli, A. Sacco et al.J. Sci Food Agric. 83 (2003) 1463-1468

IRMS = Isotopic Ratio Mass Spectrometry

29

# Spettri NMR HRMAS - Matrici alimentari

Farine



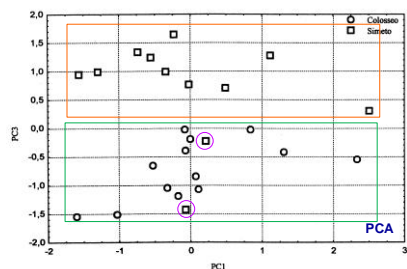
S. Ghelli, A. Sacco et al.J. Cereal Chem. 79(2), (2002) 238-242  
S. Ghelli, A. Sacco et al.J. Sci Food Agric. 83 (2003) 1463-1468

DA = Discriminant Analysis

30

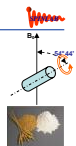
# Spettri NMR HRMAS - Matrici alimentari

## Farine



PCA  
1H-NMR  
IRMS C,O,N

S. Ghelli, A. Sacco et al. *J. Cereal Chem.* 79(2), (2002) 238-242  
S. Ghelli, A. Sacco et al. *J. Sci Food Agric.* 83 (2003) 1463-1468



# Spettri NMR HRMAS - Matrici alimentari

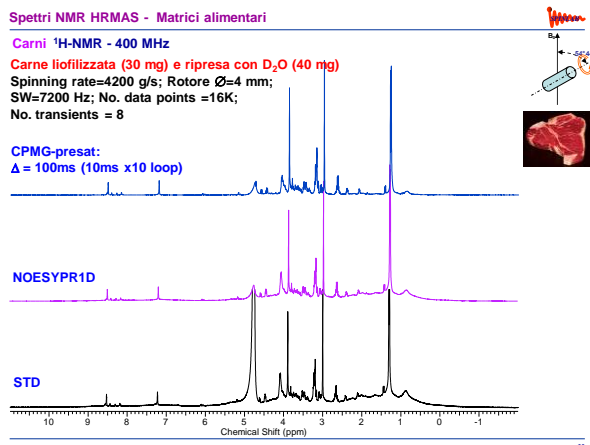
## Carni 1H-NMR - 400 MHz

**Carne liofilizzata (30 mg) e ripresa con D<sub>2</sub>O (40 mg)**  
Spinning rate=4200 g/s; Rotore Ø=4 mm;  
SW=7200 Hz; No. data points =16K;  
No. transients = 8

CPMG-preset:  
Δ = 100ms (10ms x10 loop)

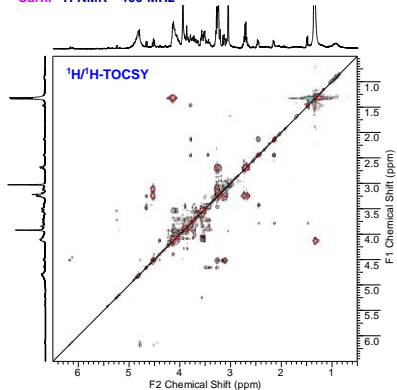
NOESYPR1D

STD



# Spettri NMR HRMAS - Matrici alimentari

## Carni 1H-NMR - 400 MHz



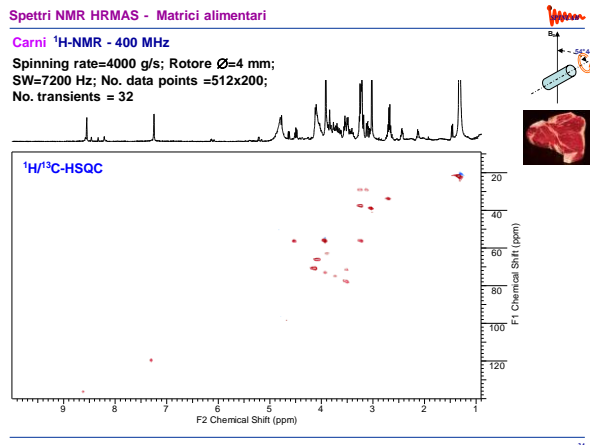
Spinning rate = 4000 g/s;  
Rotore Ø = 4 mm;  
SW=7200 Hz; No.  
data points = 1024x256;  
mixing time = 50ms;  
No. transients = 8

# Spettri NMR HRMAS - Matrici alimentari

## Carni 1H-NMR - 400 MHz

Spinning rate=4000 g/s; Rotore Ø=4 mm;  
SW=7200 Hz; No. data points =512x200;  
No. transients = 32

1H/13C-HSQC



# Spettri NMR HRMAS - Matrici alimentari

## Carni 1H-NMR - 400 MHz

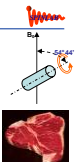
Table 1. 1H and 13C chemical shift assignment of compounds detected in a meat sample.

δ 1H (ppm)	Group	δ 13C (ppm)	Multiplicity	Compound
0.99	CH <sub>3</sub>		Doublet	Valine
1.04	CH <sub>3</sub>		Doublet	Valine
1.34	CH <sub>3</sub>	20.42	Doublet	Lactate
1.48	CH <sub>3</sub>	16.61	Doublet	Alanine
2.14	β-CH <sub>2</sub>	27.13	Multiplet	Glutamine
2.45	γ-CH <sub>2</sub>	31.70	Multiplet	Glutamine
2.70	CH <sub>2</sub>	32.50	Multiplet	Carnosine
3.04	CH <sub>2</sub>	37.71	Singlet	Creatine
3.15	CH <sub>2</sub>	27.76	Multiplet	Carnosine
3.25	C2H	74.72	Multiplet	β-glucose
3.30	CH	27.76	Multiplet	Carnosine
3.40	C4H	70.16	Multiplet	β-glucose
3.42	C4H	70.24	Multiplet	α-glucose
3.52	C3H, C5H	76.07	Multiplet	β-glucose

Table 1. 1H and 13C chemical shift assignment of compounds detected in a meat sample.

δ 1H (ppm)	Group	δ 13C (ppm)	Multiplicity	Compound
3.55	C2H	71.83	Multiplet	α-glucose
3.72	C3H	73.16	Multiplet	α-glucose
3.79	α-CH	54.58	Multiplet	Alanine
3.79	α-CH	54.65	Multiplet	Glutamine
3.85	CH	72.24	Multiplet	α-glucose
3.94	N-CH <sub>3</sub>	54.30	Singlet	Creatine
4.13	OH	68.89	Quartet	Lactate
4.51	OH	54.55	Multiplet	Carnosine
4.65	C1H	96.26	Doublet	β-glucose
5.25	C1H	92.53	Doublet	α-glucose
6.88	H3, H5		Doublet	Tyrosine
7.19	H2, H6		Doublet	Tyrosine
7.26	C4H	117.18	Singlet	Carnosine
8.56	C2H	133.77	Singlet	Carnosine

S. Ghelli, A. Sacco et al. *Ital. J. Animal Sci.* 1 (2002) 151-158



# Spettri NMR HRMAS - Matrici alimentari

## Carni 1H-NMR - 600 MHz

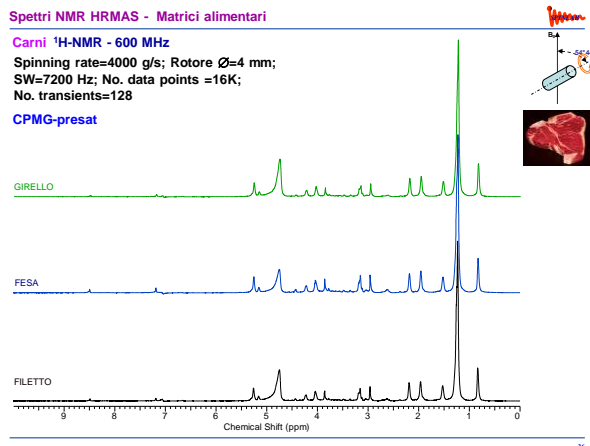
Spinning rate=4000 g/s; Rotore Ø=4 mm;  
SW=7200 Hz; No. data points =16K;  
No. transients=128

CPMG-preset

GIRELLO

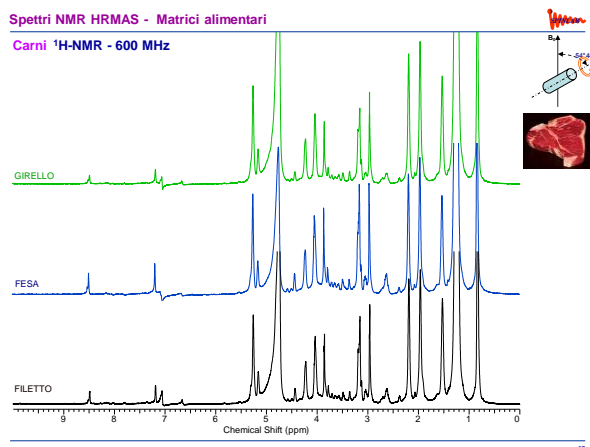
FESA

FILETTO



# Spettri NMR HRMAS - Matrici alimentari

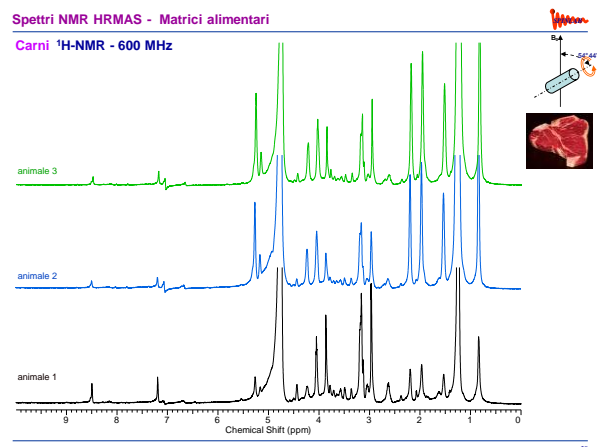
Carni <sup>1</sup>H-NMR - 600 MHz



37

# Spettri NMR HRMAS - Matrici alimentari

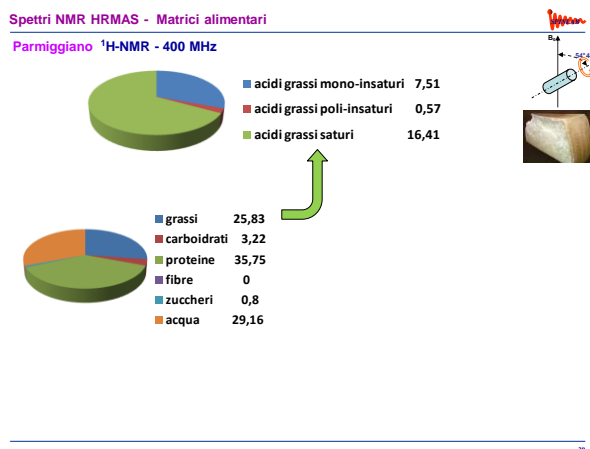
Carni <sup>1</sup>H-NMR - 600 MHz



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# Spettri NMR HRMAS - Matrici alimentari

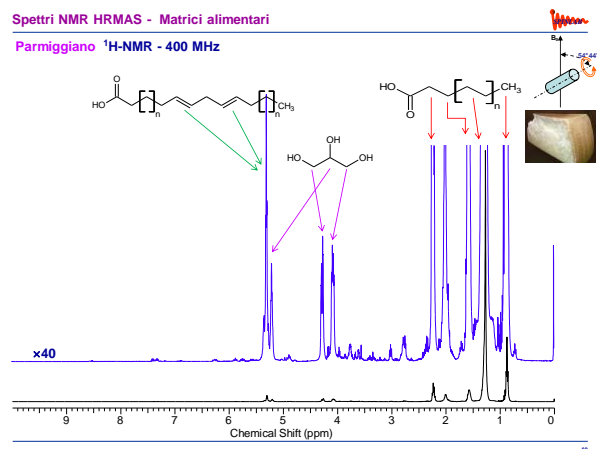
Parmiggiano <sup>1</sup>H-NMR - 400 MHz



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# Spettri NMR HRMAS - Matrici alimentari

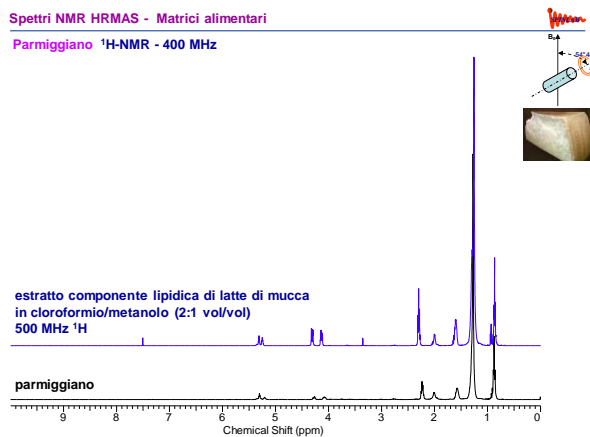
Parmiggiano <sup>1</sup>H-NMR - 400 MHz



40

# Spettri NMR HRMAS - Matrici alimentari

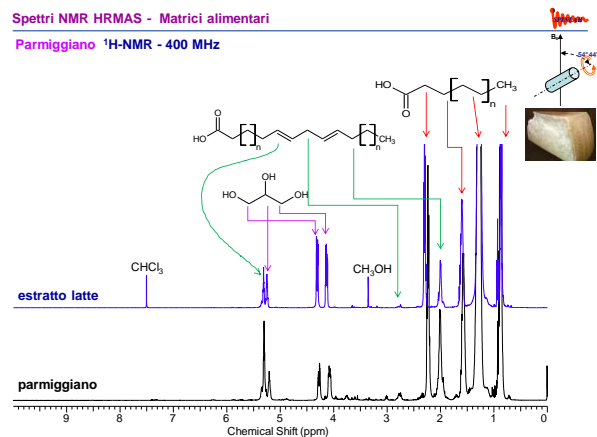
Parmiggiano <sup>1</sup>H-NMR - 400 MHz



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# Spettri NMR HRMAS - Matrici alimentari

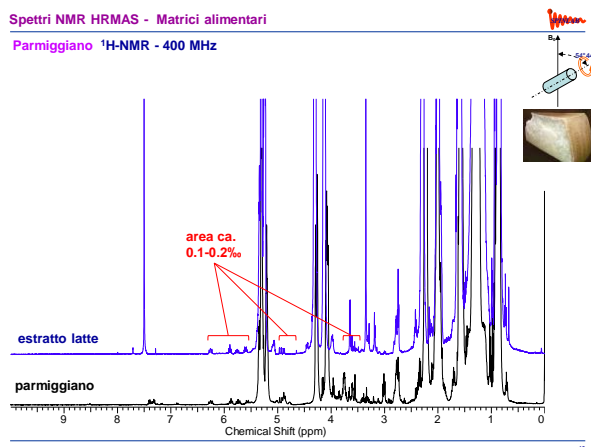
Parmiggiano <sup>1</sup>H-NMR - 400 MHz



42

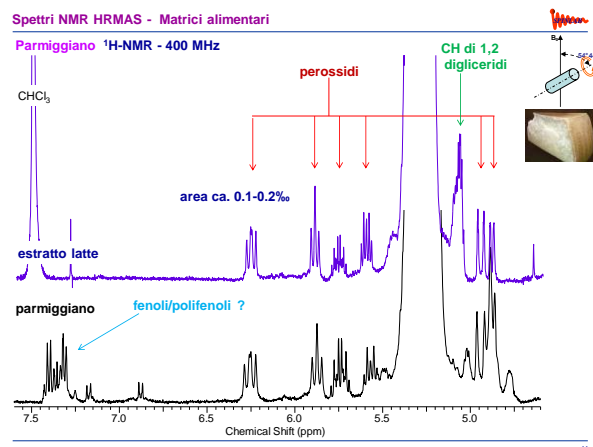
# Spettri NMR HRMAS - Matrici alimentari

Parmiggiano <sup>1</sup>H-NMR - 400 MHz



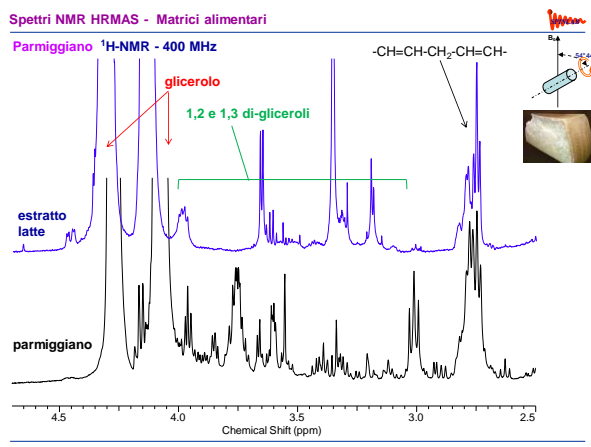
# Spettri NMR HRMAS - Matrici alimentari

Parmiggiano <sup>1</sup>H-NMR - 400 MHz



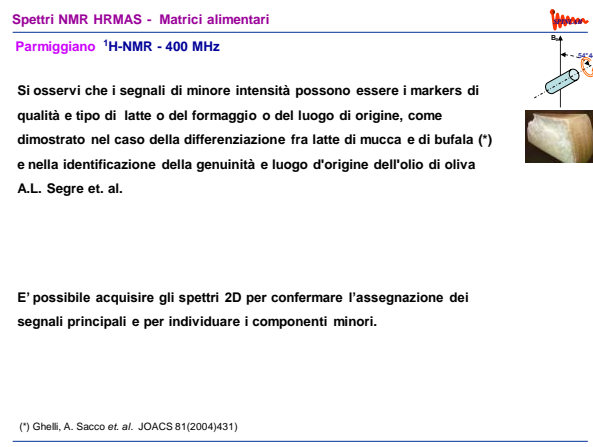
# Spettri NMR HRMAS - Matrici alimentari

Parmiggiano <sup>1</sup>H-NMR - 400 MHz



# Spettri NMR HRMAS - Matrici alimentari

Parmiggiano <sup>1</sup>H-NMR - 400 MHz



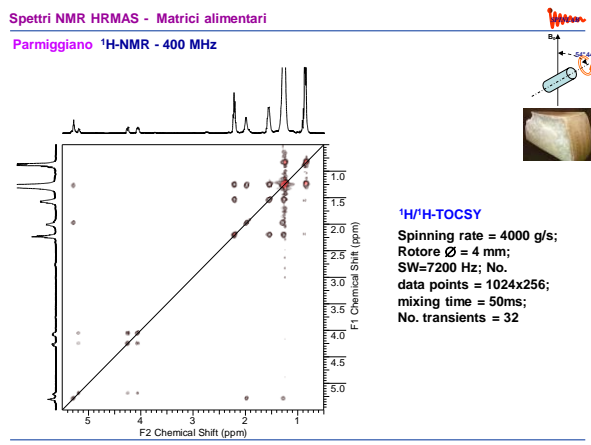
Si osservi che i segnali di minore intensità possono essere i markers di qualità e tipo di latte o del formaggio o del luogo di origine, come dimostrato nel caso della differenziazione fra latte di mucca e di bufala (\*) e nella identificazione della genuinità e luogo d'origine dell'olio di oliva A.L. Segre et. al.

E' possibile acquisire gli spettri 2D per confermare l'assegnazione dei segnali principali e per individuare i componenti minori.

(\*) Ghelli, A. Sacco et. al. JOACS 81(2004)431

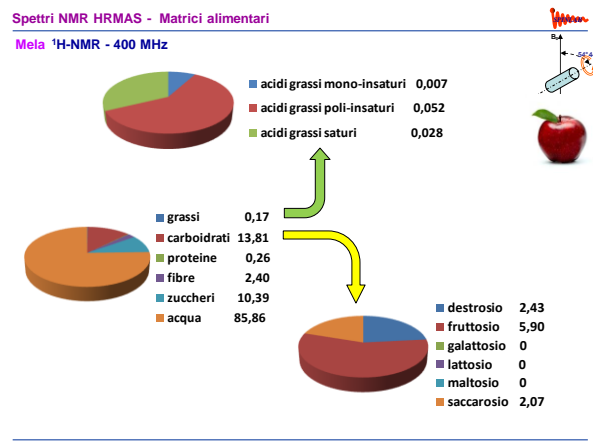
# Spettri NMR HRMAS - Matrici alimentari

Parmiggiano <sup>1</sup>H-NMR - 400 MHz



# Spettri NMR HRMAS - Matrici alimentari

Mela <sup>1</sup>H-NMR - 400 MHz

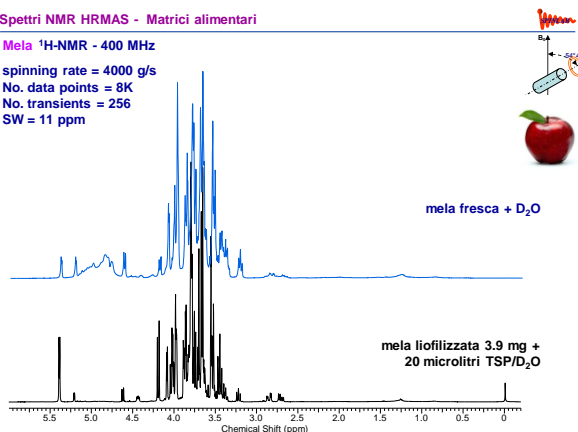




# Spettri NMR HRMAS - Matrici alimentari

## Mela <sup>1</sup>H-NMR - 400 MHz

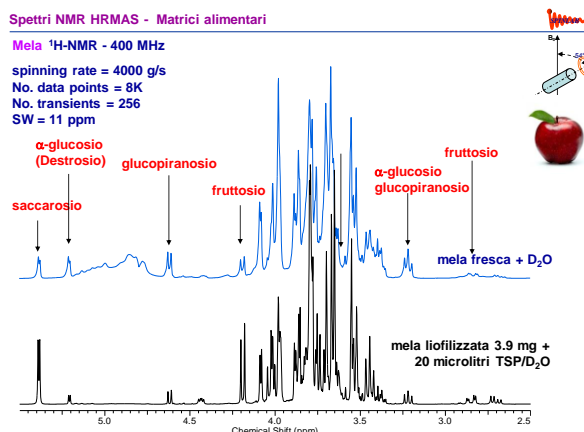
spinning rate = 4000 g/s  
No. data points = 8K  
No. transients = 256  
SW = 11 ppm



# Spettri NMR HRMAS - Matrici alimentari

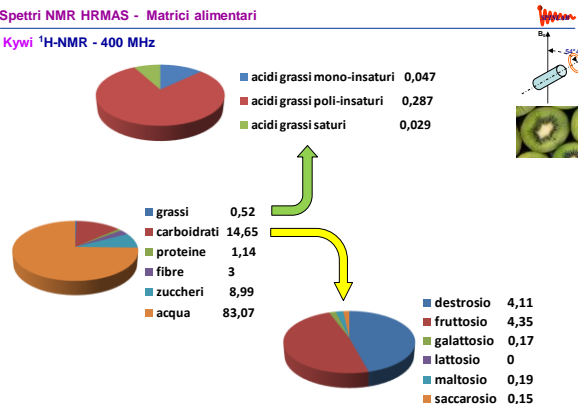
## Mela <sup>1</sup>H-NMR - 400 MHz

spinning rate = 4000 g/s  
No. data points = 8K  
No. transients = 256  
SW = 11 ppm



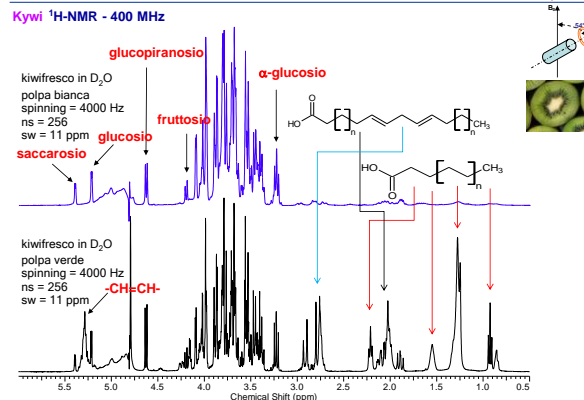
# Spettri NMR HRMAS - Matrici alimentari

## Kywi <sup>1</sup>H-NMR - 400 MHz



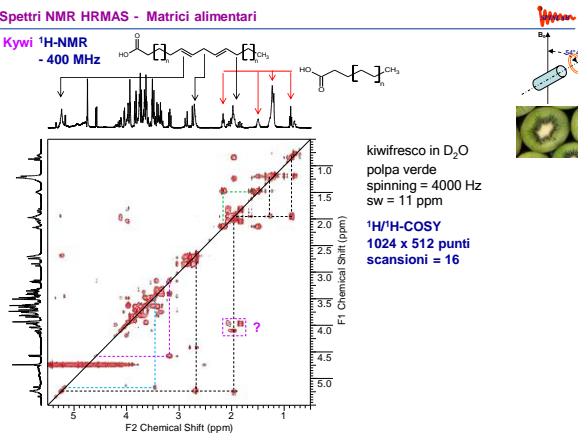
# Spettri NMR HRMAS - Matrici alimentari

## Kywi <sup>1</sup>H-NMR - 400 MHz



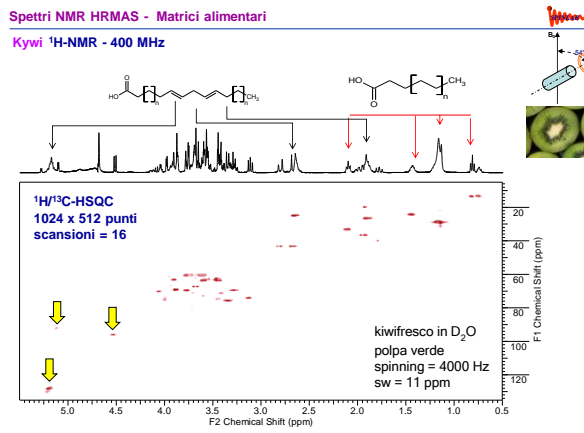
# Spettri NMR HRMAS - Matrici alimentari

## Kywi <sup>1</sup>H-NMR - 400 MHz



# Spettri NMR HRMAS - Matrici alimentari

## Kywi <sup>1</sup>H-NMR - 400 MHz



## HR-MAS NMR matrici biologiche

## HR-MAS NMR: matrici biologiche

### Fluidi biologici

- Sangue
- Plasma
- Bile
- Fluido cerebrospinale
- Fluido amniotico
- Sperma
- Fluido lacrimale
- Saliva
- Latte umano
- Fluido sinoviale
- Umor acqueo
- .....

NMR in alta  
risoluzione  
in soluzione

<sup>1</sup>H-NMR  
<sup>1</sup>H-LC-NMR  
<sup>1</sup>H-NMR-DOSY  
<sup>13</sup>C-NMR

- Studio della biochimica delle malattie
- Diagnosi precoce delle patologie
- Monitoraggio evoluzione delle patologie
- Ottimizzazione delle terapie
- Farmaco-cinetica
- Farmaco-dinamica
- Tossicologia

### Tipo di analisi

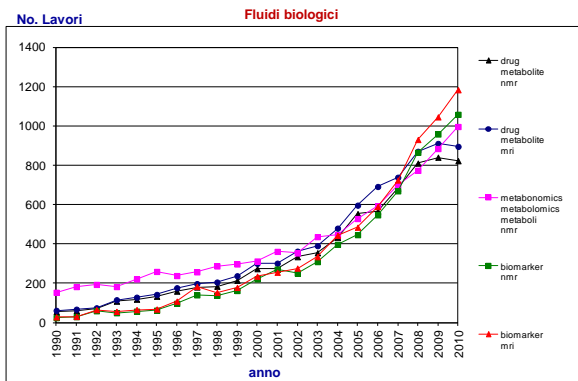
#### • Profilo metabolico

- spettri 1D
- proiezione in F1 di spettri 2D-Jresolved

#### • Ricerca di markers

- spettri 1D
- spettri 2D per riconoscimento dei metaboliti (<sup>1</sup>H/<sup>1</sup>H COSY; <sup>1</sup>H/<sup>1</sup>H TOCSY; <sup>1</sup>H/<sup>13</sup>C HSQC ecc.)

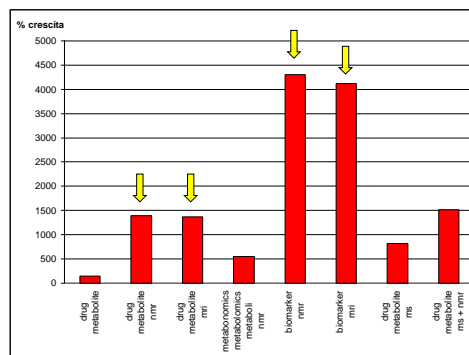
## HR-MAS NMR: matrici biologiche



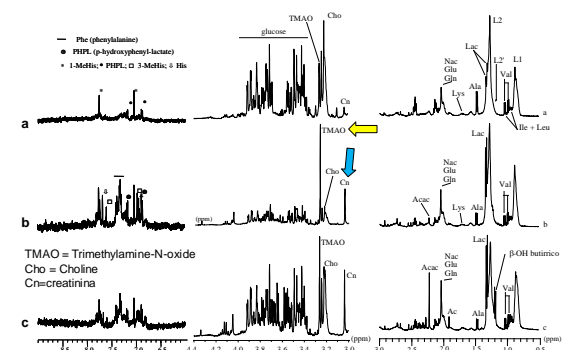
## HR-MAS NMR: matrici biologiche

No. Lavori citati su PubMed  
periodo 1990 - 2010

### Fluidi biologici



## HR-MAS NMR: matrici biologiche



## HR-MAS NMR: matrici biologiche

### Fluidi biologici

- Sangue
- Plasma
- Bile
- Fluido cerebrospinale
- Fluido amniotico
- Sperma
- Fluido lacrimale
- Saliva
- Latte umano
- Fluido sinoviale
- Umor acqueo
- .....

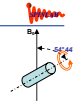
E' possibile estendere i successi  
ottenuti con i fluidi biologici  
ai tessuti biologici  
?

Si

Spettri NMR HRMAS - Matrici biologiche

Scopo dell'analisi <sup>1</sup>H-HR-MAS-NMR

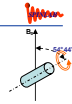
- Caratterizzazione cellulare
  - Studio della biochimica
  - Studio degli effetti delle terapie (farmacologiche & radiologiche)
  - Messa a punto di analisi in vivo <sup>1</sup>H-MRS
  - Cellular uptake
- studio delle patologie e delle relative terapie
- terapie personalizzate
- metodi non-invasivi alternativi alla biopsia e/o più affidabili delle biopsia
- sviluppo di farmaci e mezzi di contrasto per diagnostica



Spettri NMR HRMAS - Matrici biologiche

Scopo dell'analisi

- Caratterizzazione cellulare
  - Studio della biochimica
  - Studio degli effetti delle terapie (farmacologiche & radioterapie)
  - Messa a punto di analisi in vivo <sup>1</sup>H-MRS
  - Cellular uptake
- Profilo metabolico
- Ricerca di markers
- Spettri 1D
- Proiezione in F1 di spettri 2D-Jresolved
- Spettri 2D per riconoscimento dei metaboliti
- Statistica
- PCA, DA, ...



Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Metabolomic analysis - Cell and Tissue	Phytochem Anal. 2014
Human plasma	Analyst. 2014
Brain metabolite and fatty acid profiles	Neurochem Int. 2013;63(5):450-7.
Metabolomic profile of the adrenal gland	Endocr Relat Cancer. 2013;20(5):705-16.
Hypothalamic metabolic compartmentation	Front Neuroenergetics. 2013;5:6.
NMR methodologies for studying mitochondrial bioenergetics.	Methods Mol Biol. 2012;810:281-309.
Analytical approaches toward successful human cell metabolome studies by NMR spectroscopy.	Anal Chem. 2009;81(12):5023-32.
Measured changes of the metabolite profile as mesenchymal stem cells differentiate to targeted fat cells in vitro: implications for non-invasive monitoring of stem cell differentiation in vivo.	J Tissue Eng Regen Med. 2008;2(8):482-90

The aim of this work was to investigate the effects of cell handling and storage on cell integrity and <sup>1</sup>H high resolution magic angle spinning (HRMAS) NMR spectra. Three different cell types have been considered (lung tumoral amniocytes, and MG-63 osteosarcoma cells).

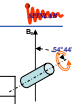


Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Patologie tumorali

Intact tumor (Paragangliomas)	J Pharm Biomed Anal. 2013.
Analysis of metabolic changes in melanoma cells after induction of melanogenesis.	Anal Biochem. 2009 Mar 15;386(2):282-4.
Study of metabolomic profiles of human esophageal carcinoma	Anal Bioanal Chem. 2013;405(10):3381-9.
Metabolomic data of human tumor tissue	J Biomed Biotechnol. 2011;2011
Lung biopsy samples	Magn Reson Med. 2014;71(1):35-43
Metabolic profiling of lung granuloma in Mycobacterium tuberculosis infected guinea pigs	J Proteome Res. 2011;10(9):4186-95.
Lung tumors	Virchows Arch. 2010 Dec;457(6):715-25.
Metabolic profiling of human lung cancer tissue	J Proteome Res. 2010 Jan;9(1):319-32.
Toward the Reliable Diagnosis of Indeterminate Thyroid Lesions: (Metabolomics Case of Study).	J Proteome Res. 2012;11(6):3317-25.
Grade classification of neuroepithelial tumors	Sci China Life Sci. 2011;54(7):606-16.
Toward improved grading of malignancy in oligodendrogliomas using metabolomics.	Magn Reson Med. 2008 May;59(5):959-65.
Metabolomic Characterization of Ovarian Epithelial Carcinomas	J Oncol. 2011;2011:174019.

HRMAS-NMR can (i) discriminate between different histological types of epithelial ovarian carcinomas and healthy ovarian tissue, (ii) generate statistical models capable of classifying borderline tumors and (iii) establish a potential relationship with patient's survival or response to chemotherapy.

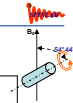


Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Patologie tumorali

Metabolic profile of intact tissue from uterine leiomyomas	NMR Biomed. 2010;23(10):1137-45
Metabolomic studies of human hepatocellular carcinoma.	J Proteome Res. 2007;6(7):2605-14
Metabolomic data of human tumor tissue	J Biomed Biotechnol. 2011;2011
Prostate cancer	NMR Biomed. 2014;27(1):90-9.
Bio-markers Related to Tumor Grade, Tumor Cell Fraction, and Cell Proliferation in Prostate Tissue.	Biomark Insights. 2011 Mar 14;6:39-47.
Detection of polyunsaturated omega-6 fatty acid in human malignant prostate	MAGMA. 2009 Dec;22(6):327-31
Metabolic assessment of cancer presence and Gleason score in human prostate needle biopsies.	MAGMA. 2008 Nov;21(6):435-42.

PSA screening has saved lives, but it has also resulted in the overtreatment of many patients with PCa because of a limited ability to accurately localize and characterize PCa lesions through imaging. High-resolution magic angle spinning (HRMAS) <sup>1</sup>H MRS has proven to be a strong potential clinical tool for PCa diagnosis and prognosis. The HRMAS technique allows valuable metabolic information to be obtained from ex vivo intact tissue samples and also enables the performance of histopathology on the same tissue specimens. Studies have found that the quantification of individual metabolite levels and metabolite ratios, as well as metabolomic profiles, shows strong potential to improve accuracy in PCa detection, diagnosis and monitoring. Ex vivo HRMAS is also a valuable tool for the interpretation of in vivo results, including the localization of tumors, and thus has the potential to improve in vivo diagnostic tests used in the clinic. Here, we primarily review publications of HRMAS <sup>1</sup>H MRS and its use for the study of intact human prostate tissue.

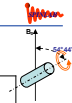


Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Tumori del cervello

Combination of high-resolution magic angle spinning proton magnetic resonance spectroscopy and microscale genomics to type brain tumor biopsies.	Int J Mol Med. 2007;20(2):199-208
Toward improved grading of malignancy in oligodendrogliomas using metabolomics.	Magn Reson Med. 2008;59(5):959-65

In spite of having been the object of considerable attention, the histopathological grading of oligodendrogliomas is still controversial. The determination of reliable biomarkers capable of improving the malignancy grading remains an essential step in working toward better therapeutic management of patients. Therefore the metabolome of 34 human brain biopsies.....



Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Tumori del cervello

Molecular classification of brain tumor biopsies	Int J Oncol. 2008 Nov;33(5):1017-25.
Ex-vivo HRMAS of adult brain tumours: metabolite quantification and assignment of tumour biomarkers	Mol Cancer. 2010;9:66.

molecular classification of brain tumors with in vivo magnetic resonance spectroscopy (MRS) is limited because of the small number of metabolites detected. In vitro MRS provides highly informative biomarker profiles at higher fields, but also consumes the sample so that it is unavailable for subsequent analysis. In contrast, ex vivo high-resolution magic angle spinning (HRMAS) MRS conserves the sample but requires large samples and can pose technical challenges for producing accurate data, depending on the sample testing temperature. We developed a novel approach that combines a two-dimensional (2D), solid-state, HRMAS proton <sup>1</sup>H NMR method, TOBSY (total through-bond spectroscopy), which maximizes the advantages of HRMAS and a robust classification strategy. We used approximately 2 mg of tissue at -8 degrees C from each of 55 brain biopsies, and reliably detected 16 different biologically relevant molecular species. We suggest that molecular characterization of brain tumors based on highly informative 2D MRS should enable us to type and prognose even inoperable patients with high accuracy in vivo.

1D and 2D 1H HRMAS NMR was used to determine that 29 small molecule metabolites, along with 8 macromolecule signals, account for the majority of the HRMAS spectrum of the main types of brain tumor (astrocytoma grade II, grade III, gliomas, lioblastomas, metastases, meningiomas and also lymphomas). Differences in concentration of 20 of these metabolites were statistically significant between these brain tumour types.



Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Tumori del cervello

Brain biopsy	J Neurosci Methods. 2011;201(1):89-97.
Metabolomic pattern of childhood neuroblastoma.	Pediatr Blood Cancer. 2011;56(1):24-34.
Ultrafast in vivo microwave irradiation for enhanced metabolic stability of brain biopsy samples during HRMAS NMR analysis.	J Neurosci Methods. 2011;201(1):89-97.
Correlations between in vivo (1)H MRS and ex vivo (1)H HRMAS metabolite measurements in adult human gliomas.	J Magn Reson Imaging. 2010;31(2):289-97
High-resolution magic angle spinning magnetic resonance spectroscopy detects glycine as a biomarker in brain tumors.	Int J Oncol. 2010;36(2):301-6
Non-invasive detection of glycine as a biomarker of malignancy in childhood brain tumours using in-vivo 1H MRS at 1.5 tesla confirmed by ex-vivo high-resolution magic-angle spinning NMR.	NMR Biomed. 2010;23(1):80-7.
human brain tumor lipids - high-resolution magic angle spinning 1H MRS and histological analysis	NMR Biomed. 2008;21(7):677-85.
Hypothalamic metabolic compartmentation during appetite regulation	Front Neuroenergetics. 2013;13:5.6.
Central neurocytoma: typical magnetic resonance spectroscopy findings and a typical ventricular dissemination.	Magn Reson Imaging. 2008;26(1):59-64



Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Effetto delle terapie

Potential Markers of Cisplatin Treatment Response Unveiled by NMR Metabolomics of Human Lung Cells.	Mol Pharm. 2013.
Therapeutic and preventive effects of methylene blue on Alzheimer's disease pathology.	Neuropharmacology. 2014;76 Pt A:68-79.
Metabonomic studies of pancreatic cancer response to radiotherapy in a mouse.	World J Gastroenterol. 2013;19(26): 4200-8.
Fluorescence-guided surgical sampling of glioblastoma identifies phenotypically distinct tumour-initiating cell populations in the tumour mass and margin.	J Cancer. 2012;107(3):462-8.

Acquiring clinically annotated, spatially stratified tissue samples from human glioblastoma (GBM) is compromised by haemorrhage, brain shift and subjective identification of 'normal' brain. We tested the use of 5-aminolevulinic acid (5-ALA) fluorescence to objective tissue sampling and to derive tumour-initiating cells (TICs) from mass and margin. METHODS: The 5-ALA was administered to 30 GBM patients. Samples were taken from the non-fluorescent necrotic core, fluorescent tumour mass and non-fluorescent margin. We compared the efficiency of isolating TICs from these areas in 5-ALA versus control patients. HRMAS (1)H NMR was used to reveal metabolic alterations due to 5-ALA. We then characterised TICs for self-renewal in vitro and tumor igenicity in vivo.



Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Effetto delle terapie

Prediction of neuroprotective treatment efficiency using a HRMAS NMR-based statistical model of refractory status epilepticus on mouse: a metabolomic approach supported by histology.	J Proteome Res. 2012;11(7):3782-95.
study metabolic responses in heart clam Ruditapes decussatus exposed to Roundup®	Talanta. 2012;97:425-31.
Study of the effect of cisplatin on the metabolic profile of MG-63 osteosarcoma cells.	J Proteome Res. 2010;9(11):5877-86
Changes in mouse brain metabolism following a convulsive dose of soman.	Toxicology. 2010 Jan 12;267(1-3):99-111.
Metabonomic studies of pancreatic cancer response to radiotherapy in a mouse xenograft model using magnetic resonance spectroscopy and principal components analysis	World J Gastroenterol. 2013;14:19(26):4200-8.
Quantitative two-dimensional HRMAS 1H-NMR spectroscopy-based metabolite profiling of human cancer cell lines and response to chemotherapy.	Magn Reson Med. 2010;63(5):1172-83.
Effects of probiotic Lactobacillus paracasei treatment on the host gut tissue metabolic profiles	J Proteome Res. 2007;6(4):1471-81
Biochemical characterization of rat intestine	J Proteome Res. 2005



Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

Studio delle terapie

Metabonomics studies of intact hepatic and renal cortical tissues from diabetic db/db mice using high-resolution magic-angle spinning 1H NMR spectroscopy.	Anal Bioanal Chem. 2009;393(6-7):1657-68.
Murine intramyocellular lipids quantified by NMR act as metabolic biomarkers in burn trauma.	Int J Mol Med. 2008;21(6):825-32.
Detection of increased scyllo-inositol in brain with magnetic resonance spectroscopy after dietary supplementation in Alzheimer's disease mouse models.	Neuropharmacology. 2010;59(4-5):353-7.
Topographical variation in metabolic signatures of human gastrointestinal biopsies	J Proteome Res. 2007;6(10):3944-51.
Longitudinal profiling of articular cartilage degradation in osteoarthritis by high-resolution magic angle spinning 1H NMR spectroscopy: experimental study in the meniscectomized guinea pig model	J Proteome Res. 2009 May;8(5):2594-600
Characterization of bovine patellar cartilage by NMR. Metabolic and structural changes in cartilage tissue are thought to be at the root of degenerative joint disease.	NMR Biomed. 2008 Mar;21(3):289-95.

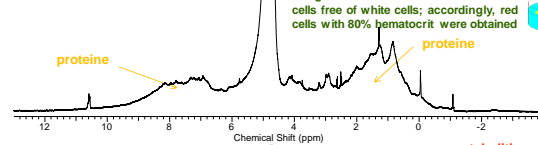


Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

1H-NMR - HRBC

600 MHz

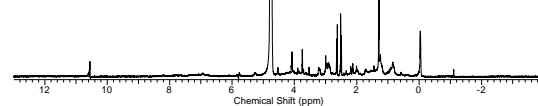
RBC 80% - ZG  
ROTORE 12ul



Living HRBC preparation: human blood (to which sodium citrate as an anticoagulant is added) was centrifuged. After that, HRBC pellets were separated from serum and white cell interface, being careful to obtain a solution of red cells free of white cells; accordingly, red cells with 80% hematocrit were obtained



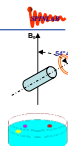
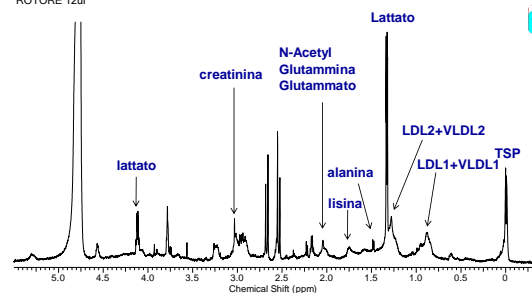
RBC 80% - CPMG\_PRESAT  
D20=100us; L1=50; SE=5 ms  
ROTORE 12ul



# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

<sup>1</sup>H-NMR - HRBC  
600 MHz

RBC 80% - CPMG\_PRESAT D2O=100u L1=50  
ROTORE 12ul



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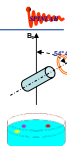
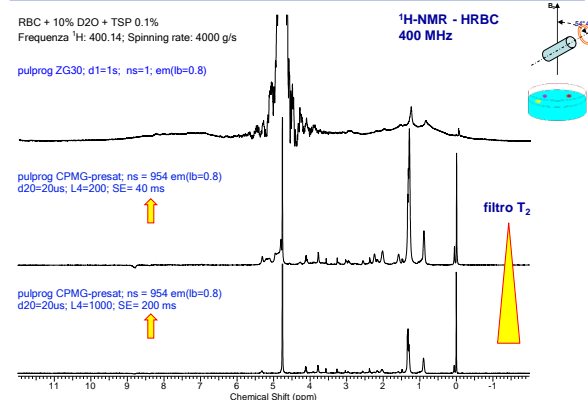
# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

RBC + 10% D2O + TSP 0.1%  
Frequenza <sup>1</sup>H: 400.14; Spinning rate: 4000 g/s

pulprog ZG30; d1=1s; ns=1; em(b=0.8)

pulprog CPMG-presat; ns = 954 em(b=0.8)  
d20=20us; L4=200; SE= 40 ms

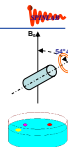
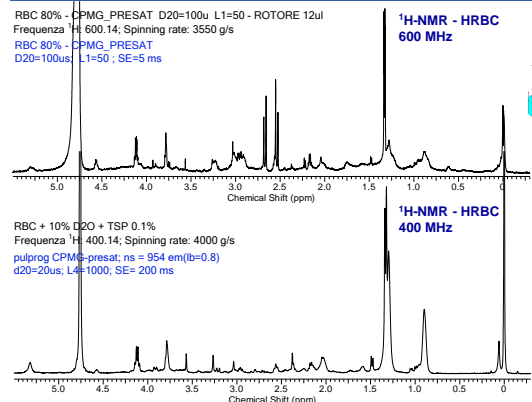
pulprog CPMG-presat; ns = 954 em(b=0.8)  
d20=20us; L4=1000; SE= 200 ms



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# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

RBC 80% - CPMG\_PRESAT D2O=100u L1=50 - ROTORE 12ul  
Frequenza <sup>1</sup>H: 600.14; Spinning rate: 3550 g/s  
RBC 80% - CPMG\_PRESAT  
D2O=100us L1=50; SE=5 ms

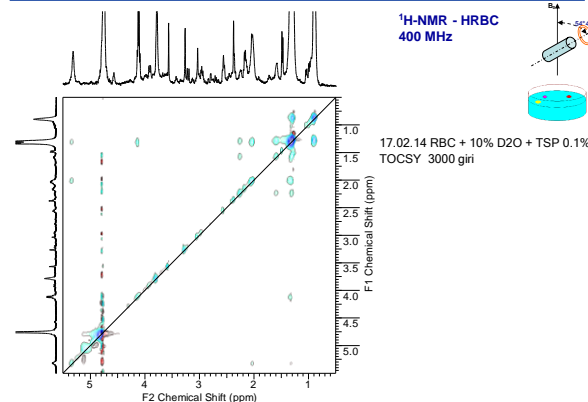


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# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

<sup>1</sup>H-NMR - HRBC  
400 MHz

17.02.14 RBC + 10% D2O + TSP 0.1%  
TOCSY 3000 giri

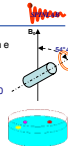
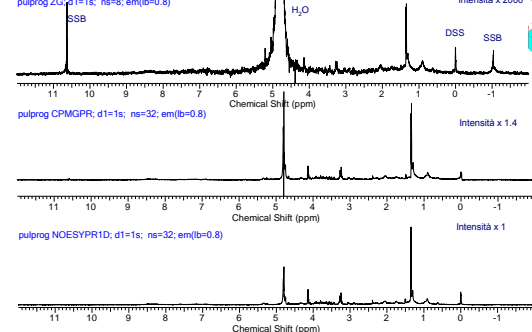


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# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

LOVO\_SC 1 10/1 monostrato di cellule, SCRAPERATE NON LAVATE DAL TERRENO - centrifugate a 2000g per 10min e "asciugate" rotore 4mm inserto sferico - 12ul di cellule - LOCK su D2O/DSS10mg/ml ext(20ul)

Frequenza <sup>1</sup>H: 600.14; Spinning rate: 3550 g/s  
pulprog ZG; d1=1s; ns=8; em(b=0.8)

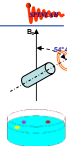
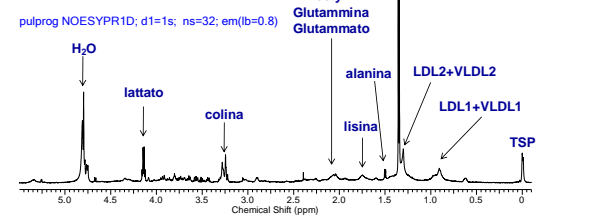


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# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

<sup>1</sup>H-NMR - HRBC  
400 MHz

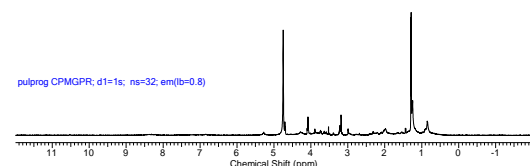
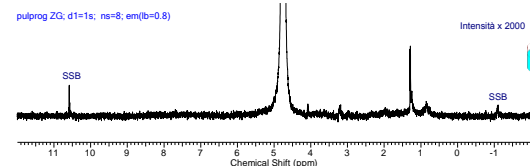
pulprog NOESYPRID; d1=1s; ns=32; em(b=0.8)



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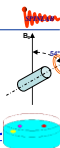
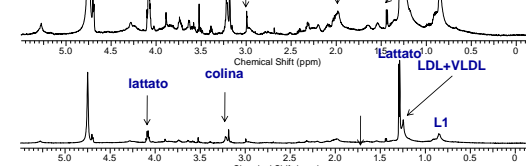
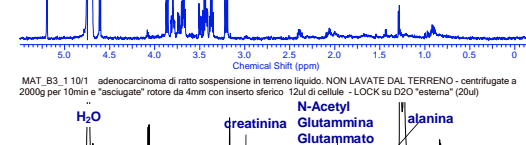
# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

MAT\_B3\_1101/1 adenocarcinoma di ratto sospensione in terreno liquido. NON LAVATE DAL TERRENO - centrifugate a 2000g per 10min e "asciugate" rotore da 4mm con inserto sferico 12ul di cellule - LOCK su D2O "esterna" (20ul)



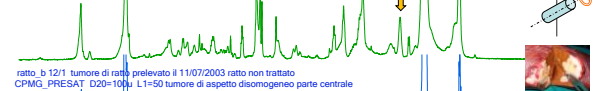
# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

MC\_COYS\_1131/1 terreno per MAT\_Bili in sospensione "fresco" rotore da 4mm con inserto sferico 12ul di campione (5ul di terreno + 5ul H2O) pulprog=ZGCPRR PL9=650B D1=3s NS=32 - LOCK su D2O "esterna" (20ul)



# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

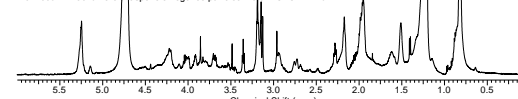
ratto\_a 12/1 tumore di ratto prelevato il 11/07/2003 ratto non trattato CPMG\_PRESEAT D2O=100u L1=50 tumore di aspetto disomogeneo parte periferica (capsula)



ratto\_b 12/1 tumore di ratto prelevato il 11/07/2003 ratto non trattato CPMG\_PRESEAT D2O=100u L1=50 tumore di aspetto disomogeneo parte centrale

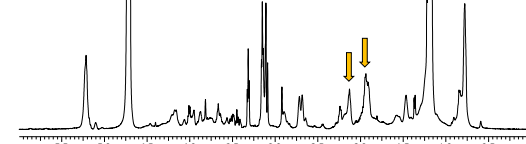
ratto\_e 12/1 tumore di ratto prelevato il 14/07/2003 ratto non trattato CPMG\_PRESEAT D2O=100u L1=50 tumore di aspetto disomogeneo parte centrale

ratto\_g 12/1 tumore di ratto prelevato il 14/07/2003 ratto non trattato CPMG\_PRESEAT D2O=100u L1=50 tumore di aspetto omogeneo parte centrale ROTORE 12ul

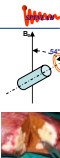
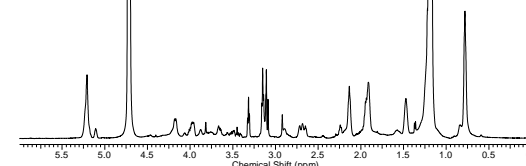


# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

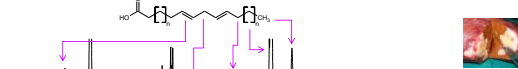
topo\_a 21/1 tumore di topo prelevato il 11/04/2003 topo N13 trattato con TAMOXIFENE CPMG\_PRESEAT L1=50 D2O=100u zona centrale del lobo con evidenti tracce di necrosi



topo\_b 21/1 tumore di topo prelevato il 11/04/2003 topo N04 CONTROLLO CPMG\_PRESEAT L1=50 D2O=100u tumore di aspetto omogeneo, non sono evidenti zone di necrosi



# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare



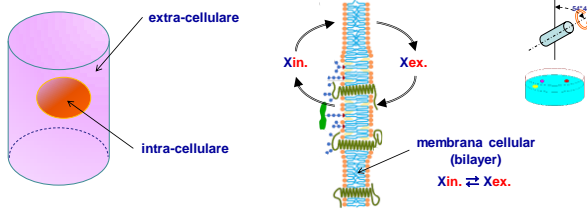
# Spettri NMR HRMAS - Matrici biologiche: Studio della biochimica cellulare

- Profilo metabolico
- Spettri 1D
- Proiezione in F1 di spettri 2D-Jresolved

- Ricerca di markers
- Spettri 1D
- Spettri 2D per riconoscimento dei metaboliti
  - <sup>1</sup>H/<sup>1</sup>H COSY
  - <sup>1</sup>H/<sup>1</sup>H TOCSY
  - <sup>1</sup>H/<sup>13</sup>C HSQC o HMQC



# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

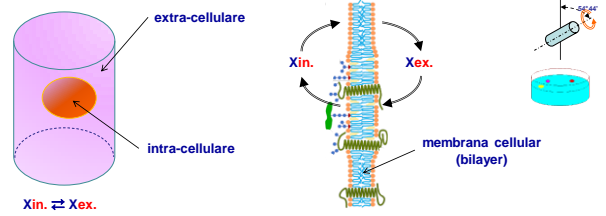


Per determinare il contenuto di  $X_{in}$  e  $X_{ex}$  di norma si procede in questo modo:

- si centrifugano le cellule
- si separano le cellule dal fluido extra-cellulare
- si analizza il fluido extra-cellulare
- si lavano le cellule e si centrifugano per eliminare il liquido di lavaggio
- si lisano le cellule
- si analizza il fluido delle cellule lisate

Essendo attivo l'equilibrio  $X_{in} \rightleftharpoons X_{ex}$  questo metodo presenta vari e rilevanti aspetti di scarsa rappresentatività e riproducibilità

# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



E' possibile studiare sistemi biologici compartimentalizzati (cellule, tessuti) senza alterare gli equilibri in corso e senza alterare le condizioni fisiologiche, cioè in vitro e/o in vivo ?

# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi non compartimentalizzati

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B$$

sistemi compartimentalizzati

$\Delta B_B$  = variazione del campo magnetico di bulk

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B \text{ (compartimento 1)}$$

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B \text{ (compartimento 2)}$$

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B \text{ (compartimento n)}$$

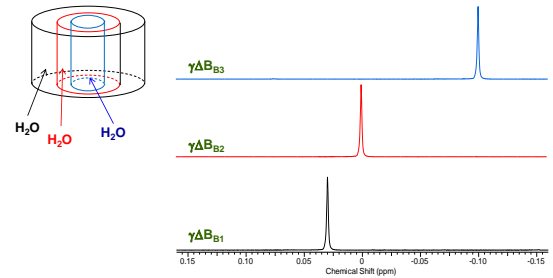
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi non compartimentalizzati

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B$$

sistemi compartimentalizzati

$\Delta B_B$  = variazione del campo magnetico di bulk



# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi non compartimentalizzati

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B$$

sistemi cellulari

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B$$



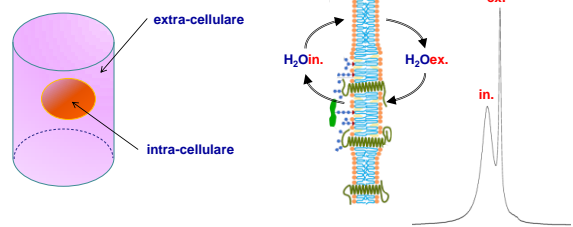
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi non compartimentalizzati

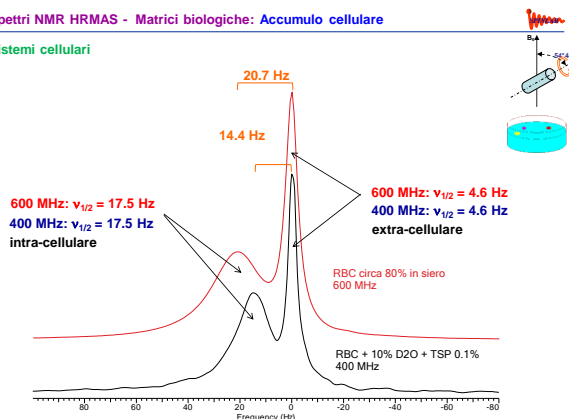
$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B$$

sistemi cellulari

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_C + \gamma \Delta B_P + \gamma \Delta B_B$$

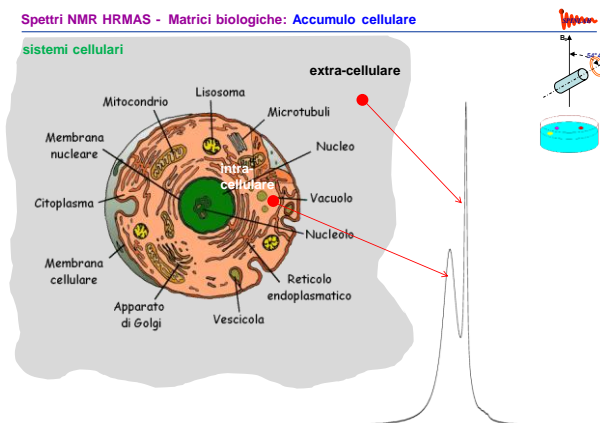


## sistemi cellulari



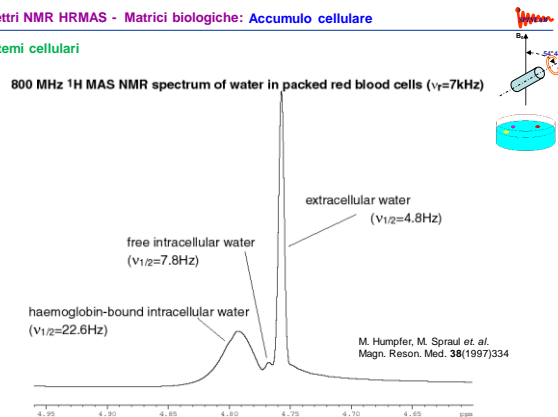
91

sistemi cellulari



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sistemi cellulari

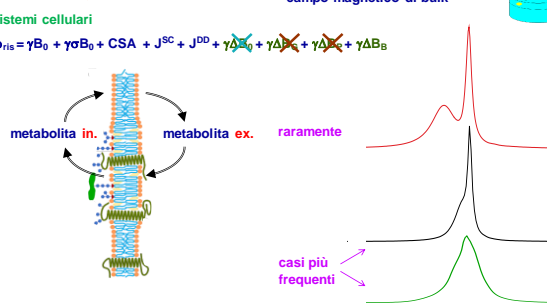


2

sistemi non compartimentalizzati

$$\omega_{\text{ris}} = \gamma B_0 + \gamma \sigma B_0 + \text{CSA} + J^{\text{SC}} + J^{\text{DD}} + \cancel{\gamma \Delta B_0} + \cancel{\gamma \Delta B_G} + \cancel{\gamma \Delta B_P} + \cancel{\gamma \Delta B_B}$$

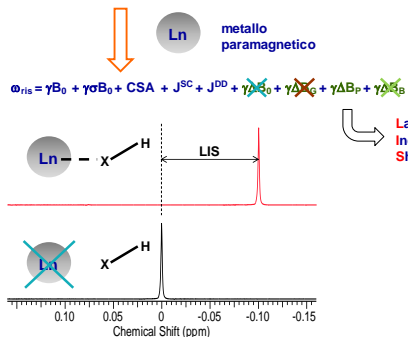
$\Delta B_B$  = variazione del campo magnetico di bulk



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**sistemi non compartimentalizzati**

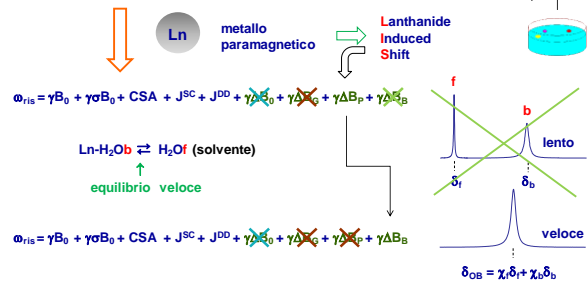
$$\omega_{\text{ris}} = \gamma B_0 + \gamma \sigma B_0 + \text{CSA} + J^{\text{SC}} + J^{\text{DD}} + \cancel{\gamma \Delta B_0} + \cancel{\gamma \Delta B_G} + \cancel{\gamma \Delta B_P} + \cancel{\gamma \Delta B_B}$$



01

**sistemi non compartimentalizzati in acqua**

$$\omega_{\text{ris}} = \gamma B_0 + \gamma \sigma B_0 + \text{CSA} + J^{\text{SC}} + J^{\text{DD}} + \cancel{\gamma B_0} + \cancel{\gamma \sigma B_0} + \cancel{\gamma \sigma B_P} + \cancel{\gamma \sigma B_S}$$



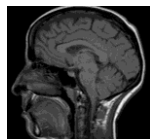
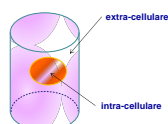
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# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

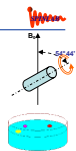
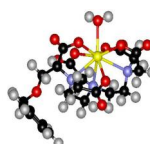
sistemi cellulari in presenza di metallo paramagnetico

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$



Magnetic Resonance Imaging

Gd-BOPTA

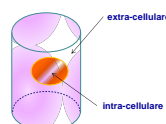


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# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi cellulari in presenza di metallo paramagnetico

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$



$\gamma \Delta B_0$  (isotropica) +  $\gamma \Delta B_1$  (anisotropica)

$$\omega_{ris} = \dots + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$

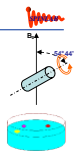
larghezza di riga più stretta

LIS minimo misurabile molto piccolo (pochi Hz)

hyperfine contact shift  
hyperfine dipolar shift

LIS solo nel compartimento ove è presente il Ln

$$LIS \propto [Ln]$$

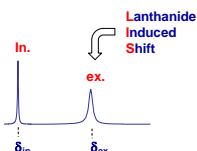
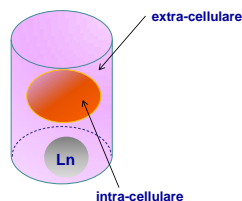


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# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi cellulari in presenza di metallo paramagnetico

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$

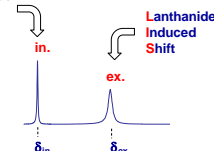
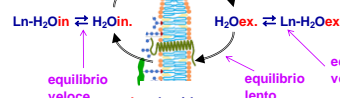
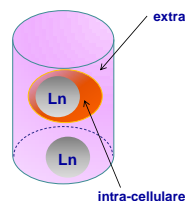


99

# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi cellulari in presenza di metallo paramagnetico

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$

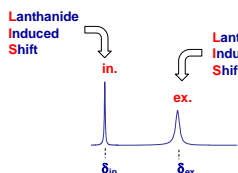


100

# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi cellulari in presenza di metallo paramagnetico

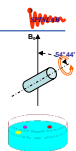
$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$



$$LIS \propto [Ln]$$

- Il segnale dell'acqua soggetto a LIS è nel comparto contenente il lantanide
- Il valore di LIS fornisce la concentrazione di lantanide presente nel comparto cellulare

Il segnale dell'acqua consente di determinare quantitativamente, in modo non-invasivo e senza modificare i sistemi all'equilibrio, l'accumulo cellulare di mezzi di contrasto (complessi di gadolinio) per Risonanza Magnetica per Immagini (MRI-CA)



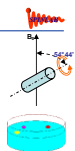
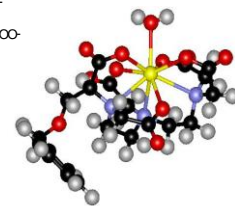
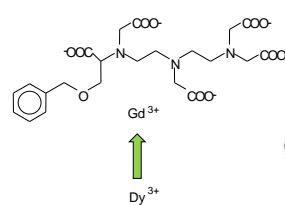
101

# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi cellulari in presenza di metallo paramagnetico

$$\omega_{ris} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{SC} + J^{DD} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$

Gd-BOPTA



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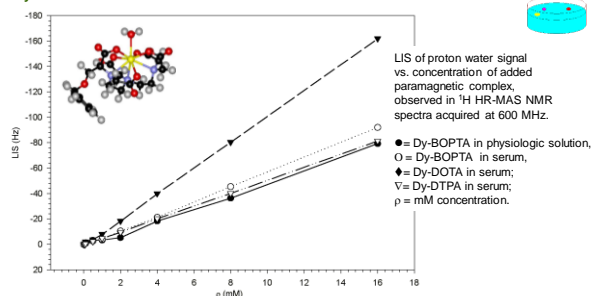
S. Ghelli, S. Calabi et al. JMR 156, 222-229 (2002)

# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare

sistemi cellulari in presenza di metallo paramagnetico

$$\omega_{\text{ris}} = \gamma B_0 + \gamma \sigma B_0 + CSA + J^{\text{SC}} + J^{\text{DD}} + \gamma \Delta B_0 + \gamma \Delta B_1 + \gamma \Delta B_2 + \gamma \Delta B_3$$

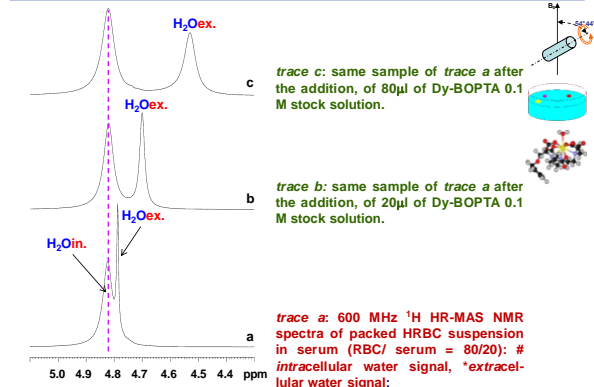
## Dy-BOPTA



S. Ghelli, S. Calabi et al. JMR 156, 222-229 (2002)

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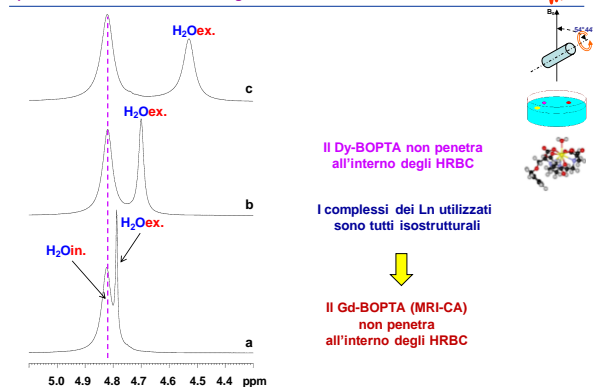
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



S. Ghelli, S. Calabi et al. JMR 156, 222-229 (2002)

184

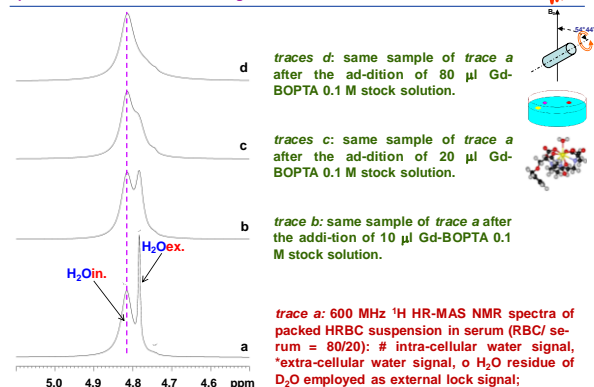
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



S. Ghelli, S. Calabi et al. JMR 156, 222-229 (2002)

185

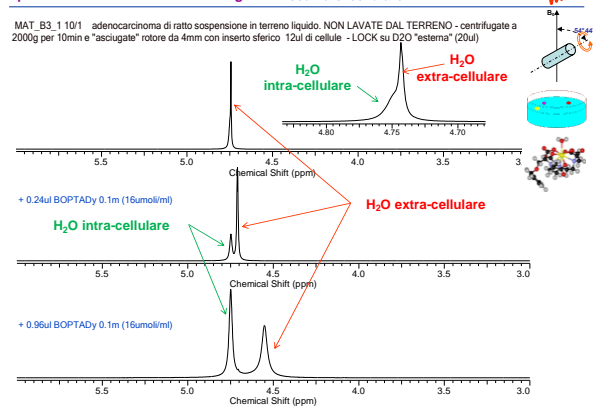
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



S. Ghelli, S. Calabi et al. JMR 156, 222-229 (2002)

186

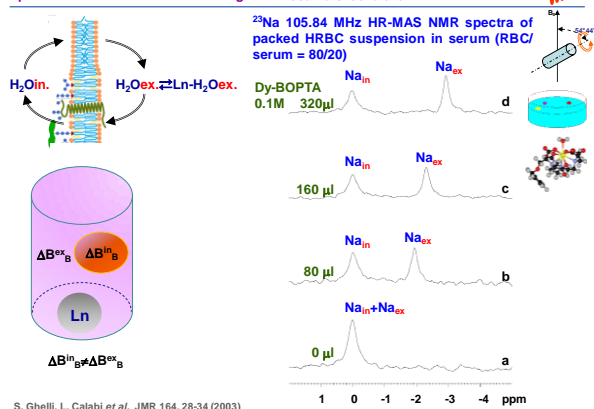
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



S. Ghelli, S. Calabi et al. JMR 164, 28-34 (2003)

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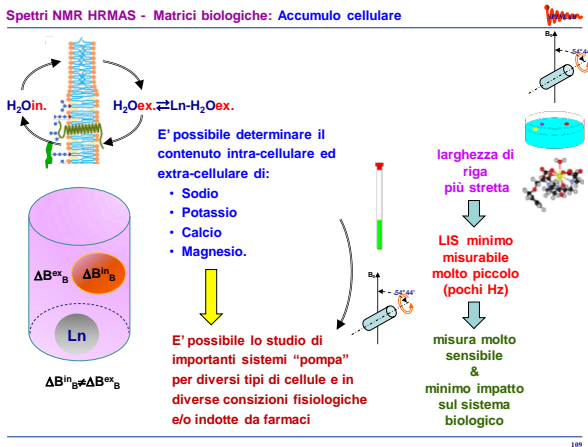
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



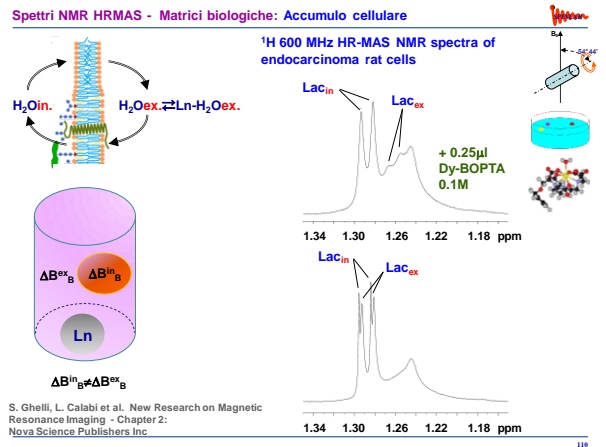
S. Ghelli, S. Calabi et al. JMR 164, 28-34 (2003)

188

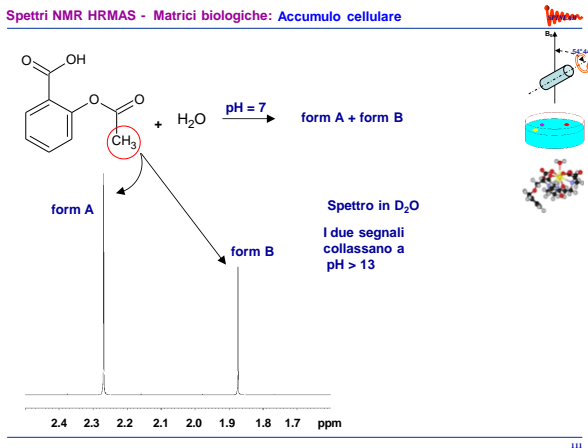
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



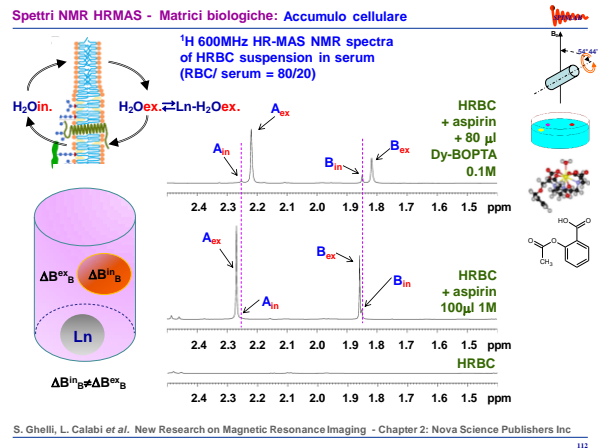
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



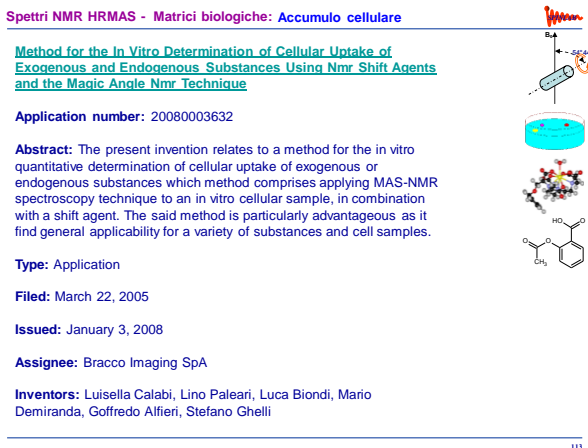
# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare



# Spettri NMR HRMAS - Matrici biologiche: Accumulo cellulare





Da stime del WHO (World Health Organization) in paesi siti in:

- Centro Africa
- Est asiatico (Cambogia, Indonesia, Cina, Korea,.....)
- Sud America (Brasile, .....)

**Il 50 % dei farmaci in commercio è contraffatto**

in paesi siti in:

- Europa (Polonia, .....)
- USA
- Canada
- Giappone

**Il 1-5 % dei farmaci in commercio è contraffatto**

E i farmaci venduti via internet

**Il 50 % sono contraffatti**

115

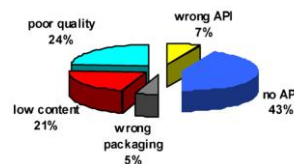


Fig. 2. WHO estimation of five categories of counterfeits.

I farmaci interessati dal fenomeno sono prevalentemente:

- prodotti per migliorare le prestazioni sessuali (sia maschili che femminili)
- prodotti per la terapia per AIDS
- prodotti salvavita (antibiotici, antimalarici, ecc....)
- integratori vitaminici & similari

Esiste quindi una grande richiesta di controlli sulla qualità dei prodotti in commercio. Ciò significa lavorare su prodotti formulati tali quali.

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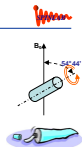
Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Scopo dell'analisi

- Caratterizzazione della composizione del preparato
- Studio della stabilità nel tempo del prodotto
- Caratterizzazione dei prodotti di degradazione

Tipo di analisi

- Spettri 1D
- Spettri 2D

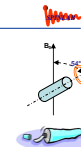


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Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Lasoactive® 10% gel

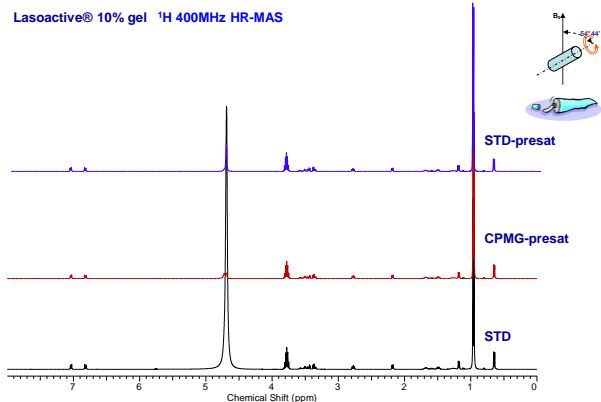
- **ibuprofene lisina**
- isopropanolo
- idrossietilcellulosa
- sodio para-ossibenzoato di metile
- sodio para-ossibenzoato di etile
- glicerolo
- lavanda essenza
- acqua



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Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

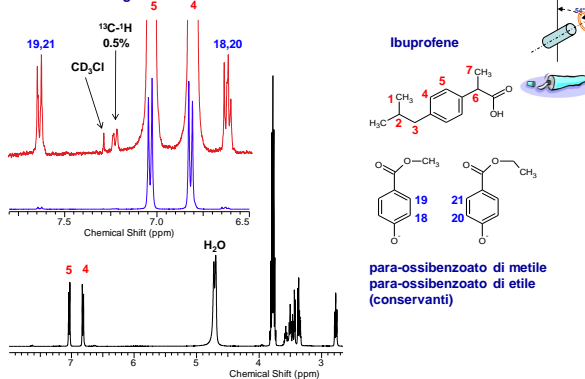
Lasoactive® 10% gel <sup>1</sup>H 400MHz HR-MAS



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Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

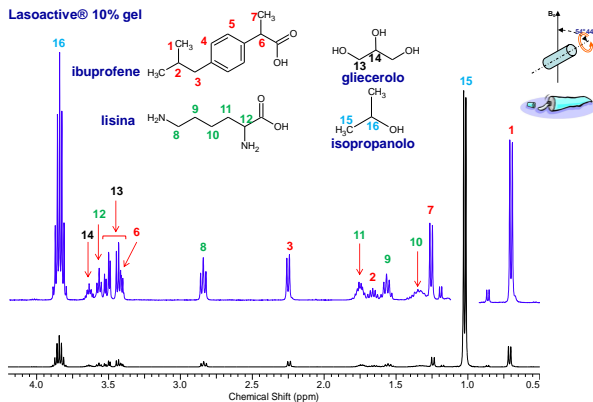
Lasoactive® 10% gel



120

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

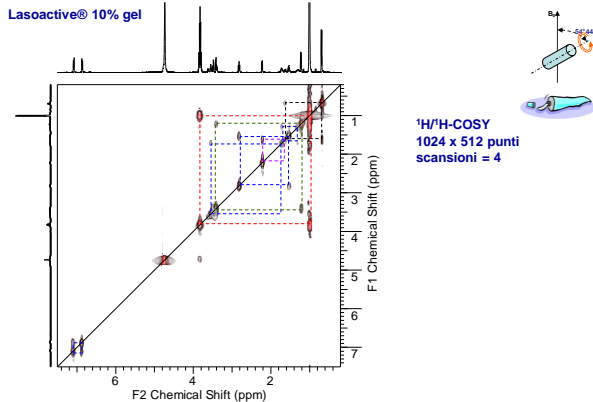
Lasoactive® 10% gel



121

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Lasoactive® 10% gel



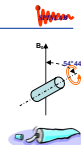
122

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Gentalyn® 0.1%

- gentamicina solfato 0.1%
- paraffina liquida
- zaselina bianca

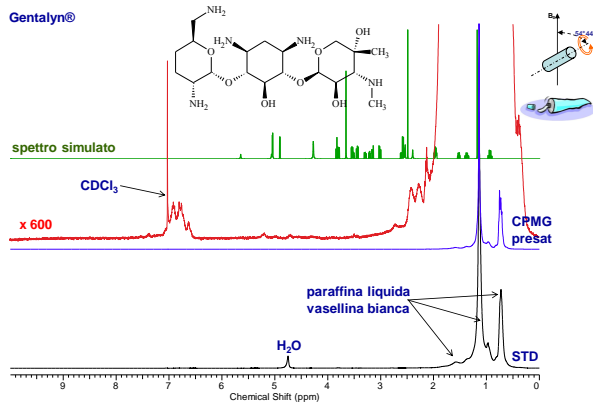
Prodotto scaduto



123

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Gentalyn®

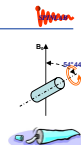


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Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Locoidon® 0.1%

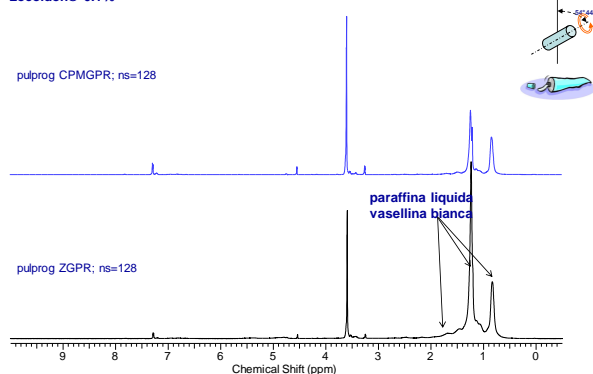
- idrocortisone 17-butilato 0.1%
- alcooli cetilsteirilico
- macrogol 25 cetostearile etere
- olio di vasellina leggero
- vasellina filante
- alcooli benzilico
- acido citrico anidro
- sodio citrato anidro
- propilene para-idrossibenzoato
- acqua depurata.



125

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

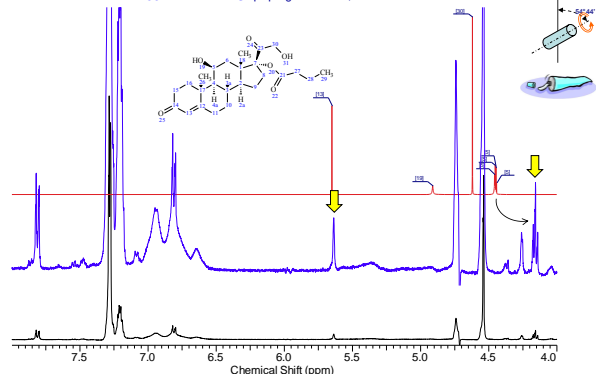
Locoidon® 0.1%



126

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

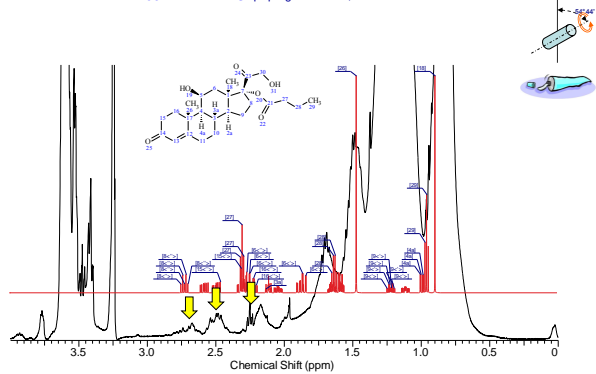
Locoidon® 0.1% <sup>1</sup>H 400MHz HR-MAS pulprog CPMGPR; ns=128



127

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Locoidon® 0.1% <sup>1</sup>H 400MHz HR-MAS pulprog CPMGPR; ns=128

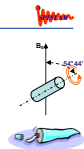


128

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Eucerin® (crema protettiva per il derma della mani)

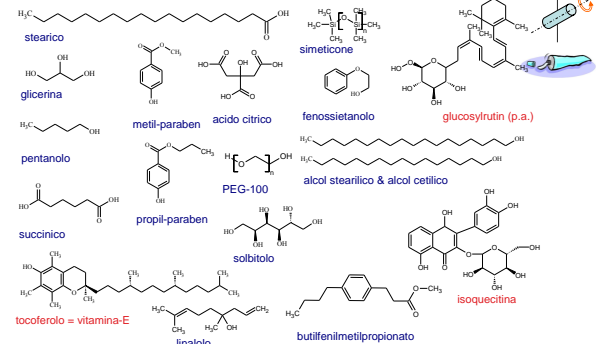
- Acqua
- glicerina
- pentanolo
- idrogenati-coco-gliceridi
- alcol stearilico (C18-OH)
- acido stearico (C17-COOH)
- alcol cetilico (C16-OH)
- amido octenilsuccinato
- simeticone
- fenossietanolo
- tocoferolo
- PEG-100
- Stearato
- glicerilstearato
- sorbitan stearato
- metilparaben
- EDTA
- citrato
- propilparaben
- acido citrico
- glucosylrutin
- isoquercitina
- linalolo
- butilfenilmetilpropionato



129

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

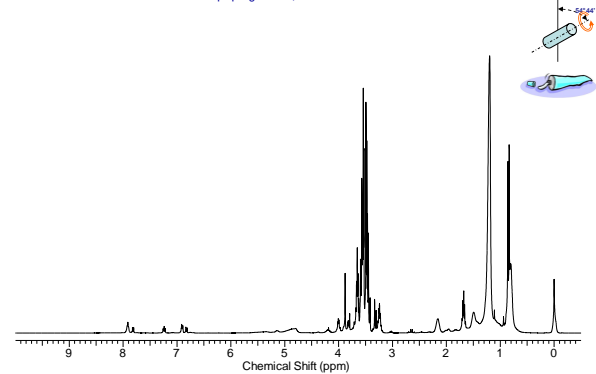
Eucerin® (crema protettiva per il derma della mani)



130

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

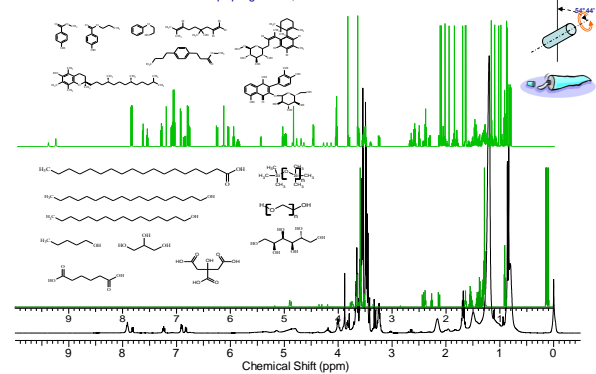
Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



131

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

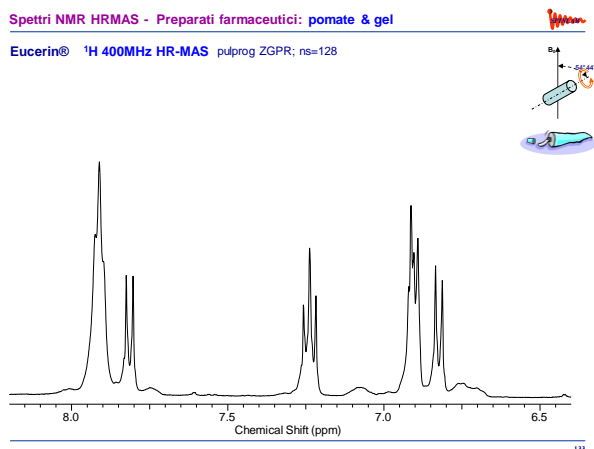
Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



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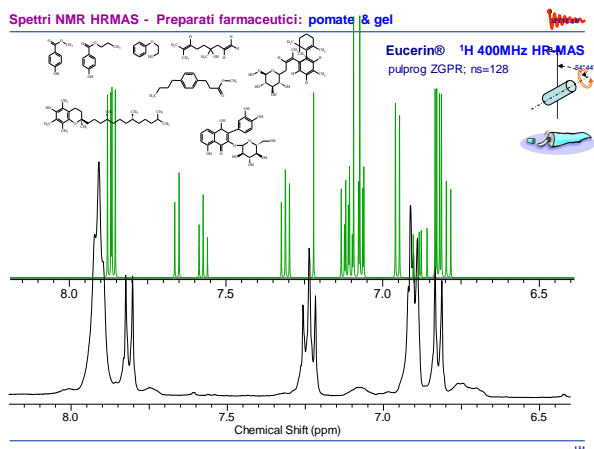
Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



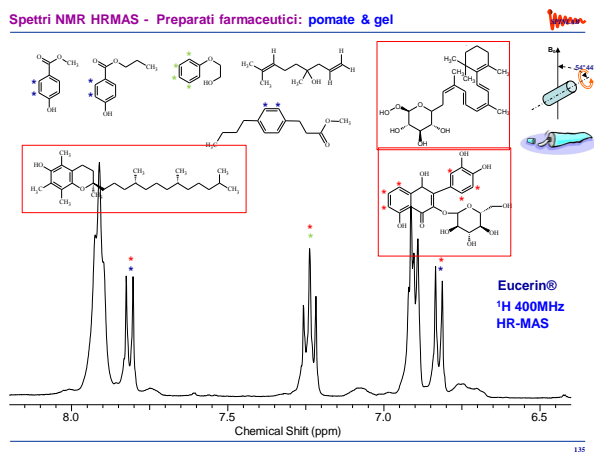
Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



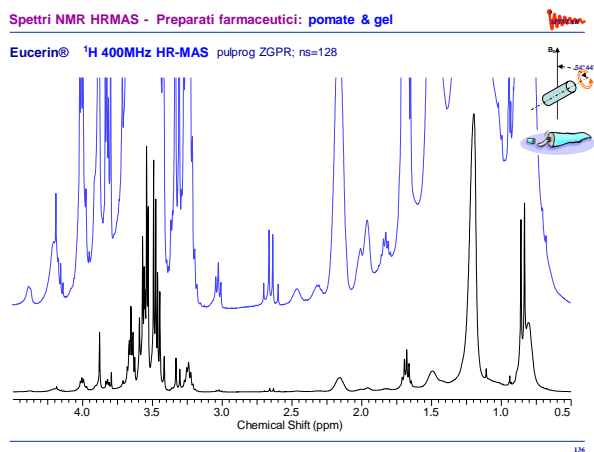
Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



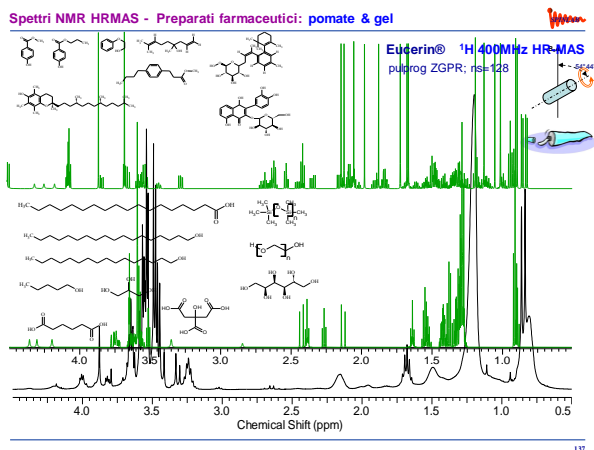
Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



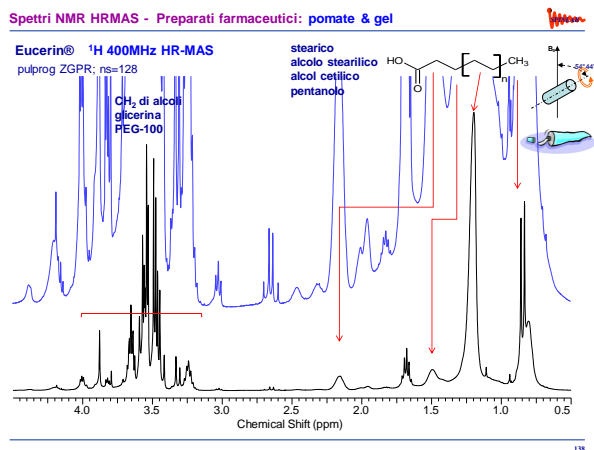
Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

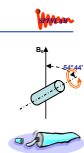
Eucerin® <sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128



# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Lenus®

- Aqua,
- C12-20 Acid PEG-8 Ester
- Urea Alcohol denat.
- Glycerin
- Cetearyl Ethylhexanoate
- Laureth 9
- Propylene Glicol
- Isostearyl Avocavate
- Panthenol
- Allantoin
- Fucus Vesiculosus
- Menthyl Laccate
- Glicine
- Maris Sal
- Laminaria Digitata
- Tocopherl Acetate
- PPG 26 Buteth 26
- PEG 40 Hydrogenated
- Castor Oil
- Triethanolamine
- Carbomer
- Isopropyl Isostearate
- BHT
- Dimethicone Cyclopentasiloxane
- Phenoxyethanol Propylparaben
- Methylparaben
- Potassium Sorbate
- Sodium Benzoate
- Ethylparaben
- Isobutylparaben
- Butylparaben

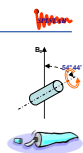
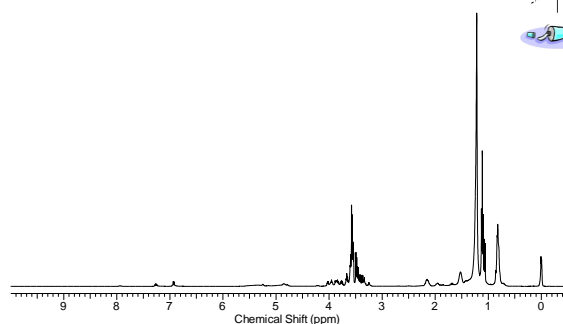


139

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Lenus®

<sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128

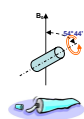
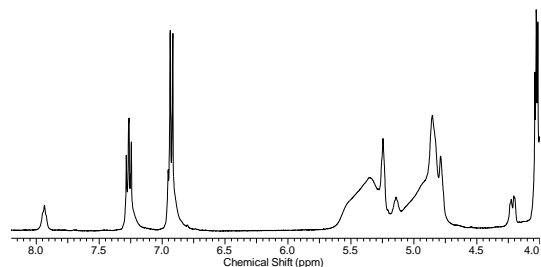


140

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

Lenus®

<sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128

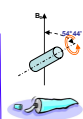
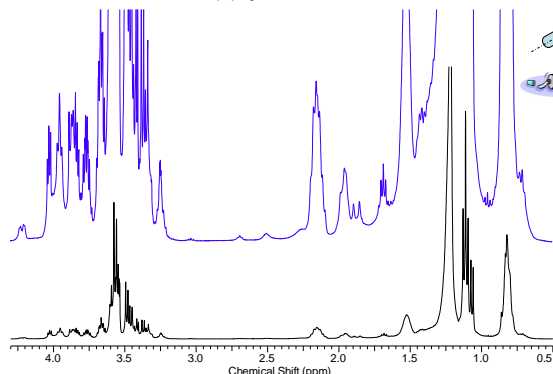


141

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

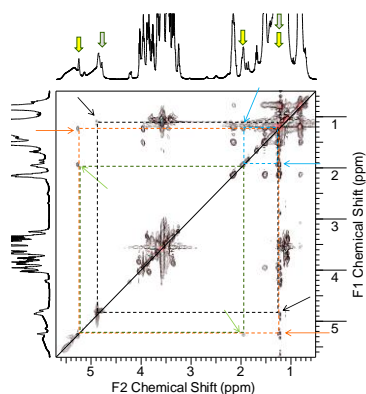
Lenus®

<sup>1</sup>H 400MHz HR-MAS pulprog ZGPR; ns=128

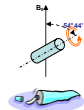


142

# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

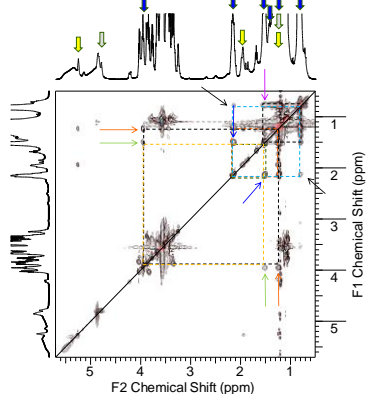


<sup>1</sup>H/<sup>1</sup>H-TOCSY  
1024 x 256 punti  
scansioni = 32

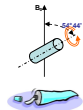


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# Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel



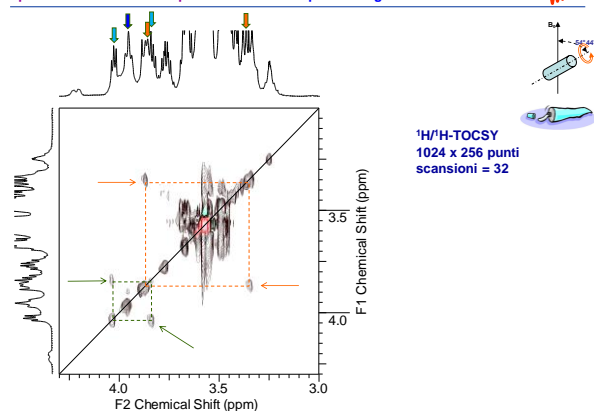
<sup>1</sup>H/<sup>1</sup>H-TOCSY  
1024 x 256 punti  
scansioni = 32



144



Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel



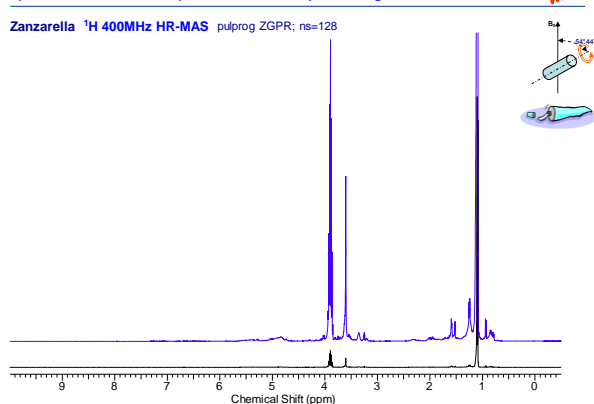
145

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

- Zanzarella - cremagel
- Aqua,
  - Isopropil alcol
  - Cymbopogon schoenathus
  - PEG-40 hydrogenated castor oil
  - Styrene acrylates copolymer
  - Sodium lauryl sulfate
  - Octoxynol-9
  - Carbomer
  - Triethanolamine
  - Geraniol
  - Eugenol
  - Mentha piperita
  - Thymus vulgaris
  - Ocimum Basilicum

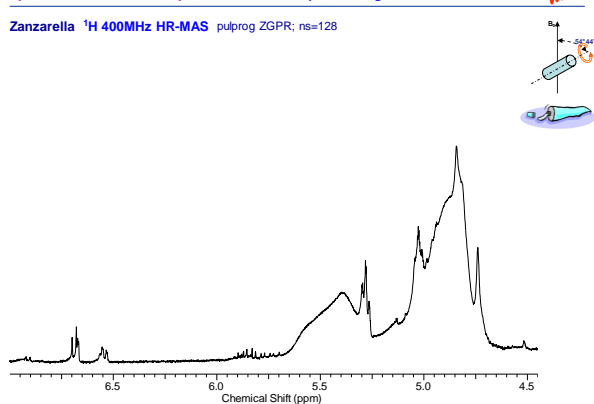
146

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel



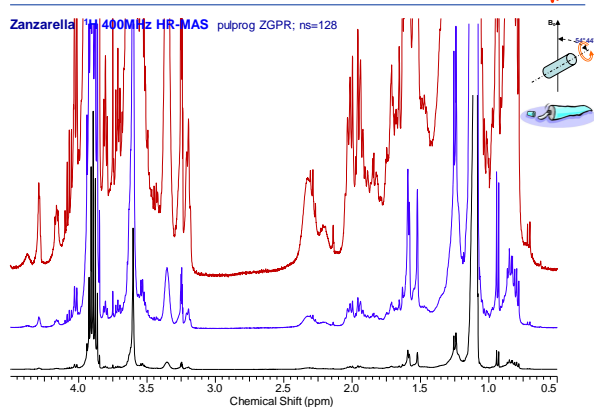
147

Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel



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Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

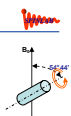


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Spettri NMR HRMAS - Preparati farmaceutici: pomate & gel

In conclusione anche nel caso di preparati farmaceutici e cosmetici, in molti casi, attraverso la HR-MAS NMR è possibile arrivare alla completa analisi del prodotto ma anche quando questo non è possibile per la complessità dello spettro, lo spettro 1D del protone può essere utilizzato come impronta digitale e, analogamente a quanto mostrato nel caso degli alimenti, attraverso l'analisi statistica dei segnali, è possibile comprendere quando il prodotto in esame è originale oppure contraffatto.

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***grazie dell'attenzione***