International Symposium “Standardisation of non-targeted methods for food authentication”,
Session II: Standardisation of Analytical Methods

Challenges in Nuclear Magnetic Resonance Spectroscopy Based Non-Targeted Analysis

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28 – 29 November 2016, Berlin

Outline

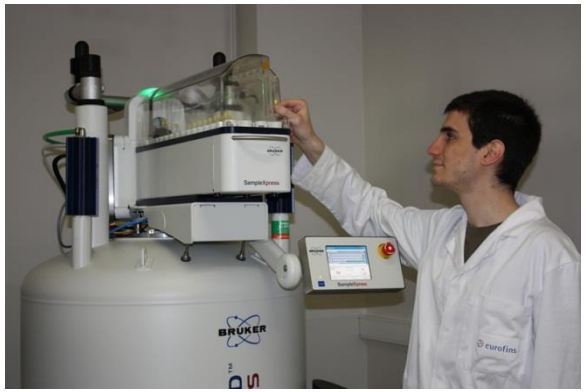
- Eurofins' experience in non-targeted testing using 1H NMR
- The advantages of using 1H NMR to ensure food authenticity
- What are the challenges to the successful standardisation of non-targeted 1H NMR
- Some solutions: what has already been achieved and what could be done in the future

Key figures
Eurofins group

- ✓ 1.95 billion € of TO
- ✓ 25,000 staff
- ✓ 39 countries
- ✓ 250 laboratories
- ✓ 130,000 analytical methods
- ✓ > 150 million assays performed each year

Specialised in food authenticity testing

Eurofins Analytics France laboratory based in Nantes (France) is the group Competence Centre for authenticity of food products



Nantes, France

Our vision of market needs

Analyses must help to enforce regulations & protect brands but:

- Need for a high number of parameters
- Often limited time, and
- Always a limited budget

- Targeted analysis efficiency is often limited
- A large part of adulterations are still undetectable

➔ Profiling 1H-NMR is a complementary tool

Eurofins experience in NMR non-targeted approach

- 4 NMR spectrometers in Nantes (2 for SNIFNMR, 2 for Profiling)
  BRUKER 400MHz with autosampler, BCU and BOSSIII
  2 qualified instruments for non-targeted approach
  2 BBI probes

- a dedicated Production Unit Profiling NMR (since 2012)

- > 5000 routine analyses in 2015
  fruit juices@, wines@, honey@, coffee@, soft drinks@, milk, spices....

@ ISO-17025 accreditation for quantification (for the moment…)

(scope available under http://www.cofrac.fr/Annexes/Sect1/1-0287.pdf)

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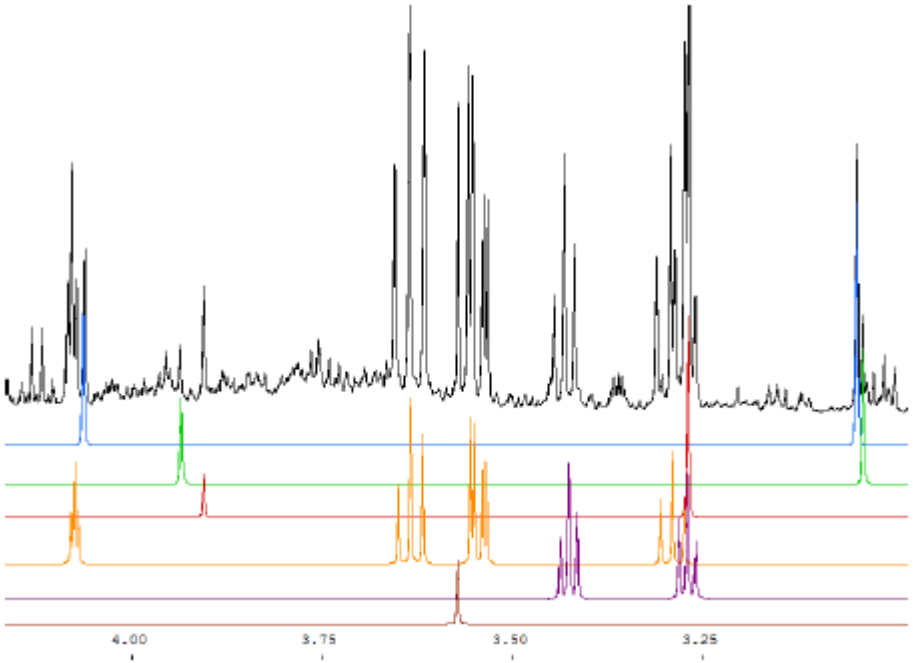
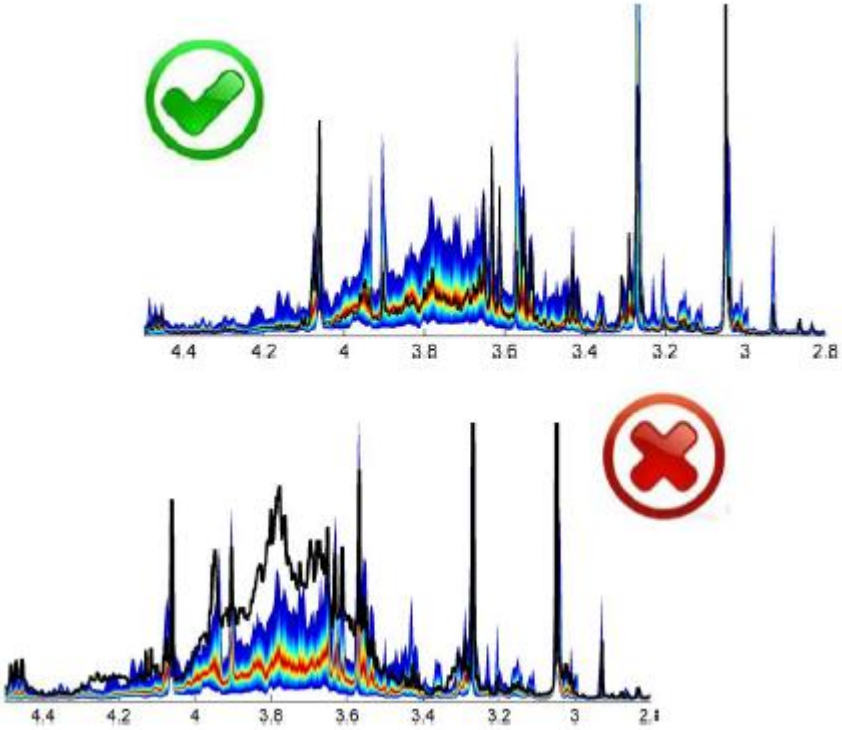
Two approaches using the same experiment

Targeted

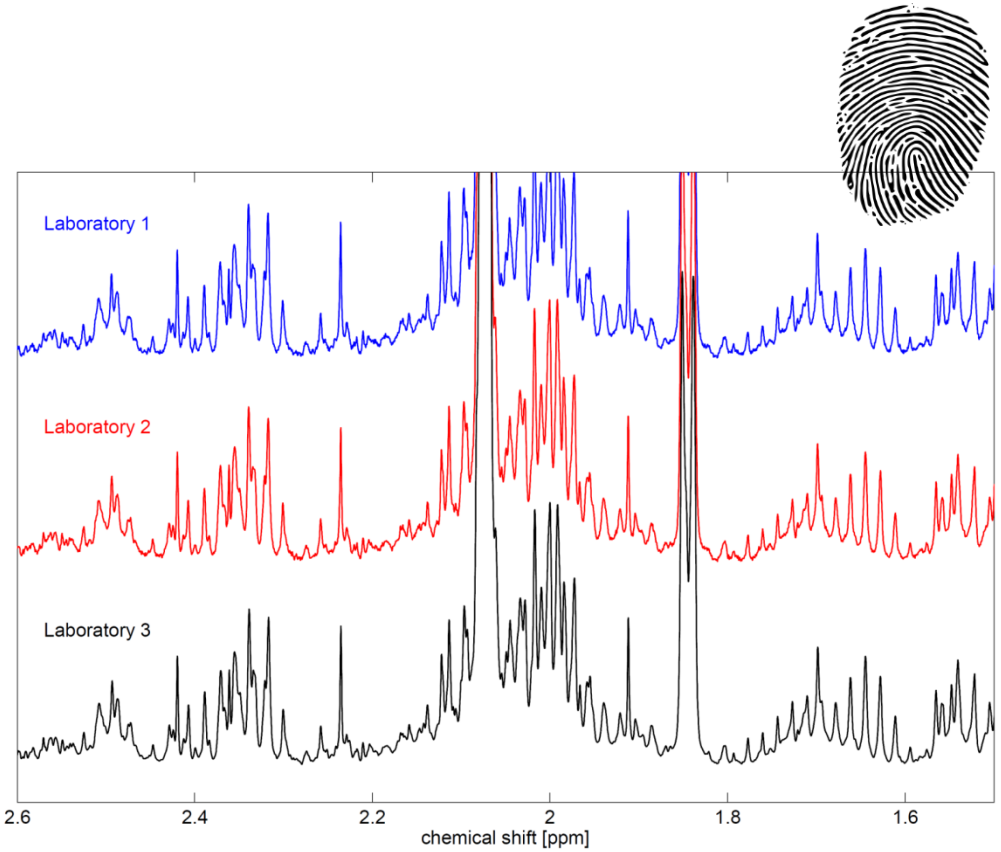


Non-targeted



Features of 1H-NMR Profiling

- Complex signal with multiple resonances for a single compound
- Primary method for quantification
  no need to calibrate each compound
- High reproducibility, even inter-laboratory
- Non-targeted detection of all protons
- 1H-NMR profile can be regarded as unique fingerprint of the sample
- Long-term build of reference databases possible
- Retrospective analysis possible
  also quantification of further compounds



Example : preparation and acquisition in 3 different labs (wine sample)

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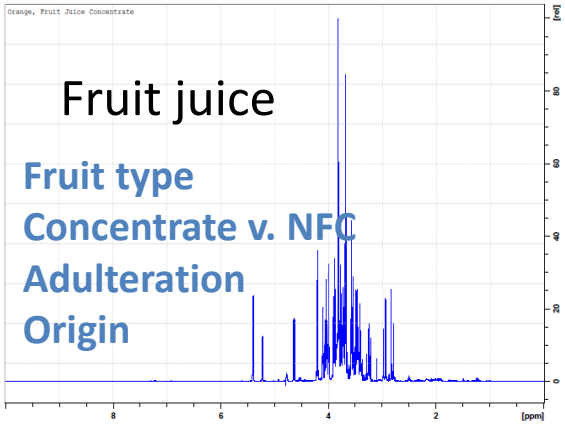
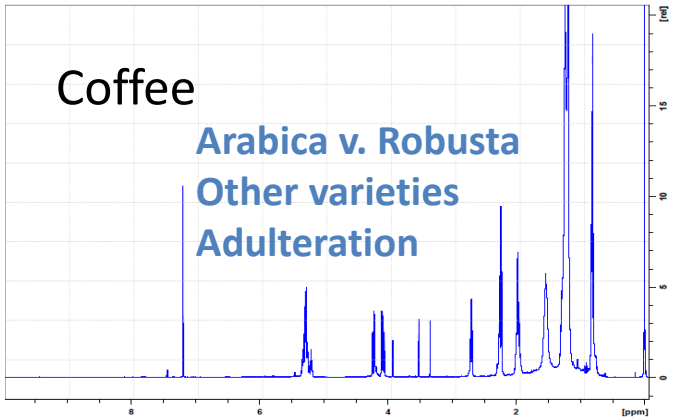
Eurofins routine applications for non-targeted 1H NMR



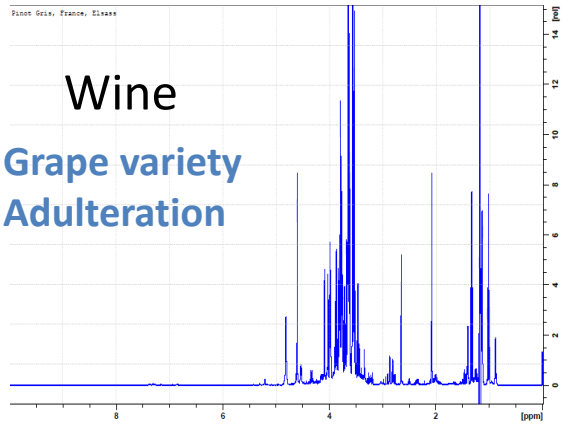
Fruit juice

Fruit type
Concentrate v. NFC
Adulteration
Origin



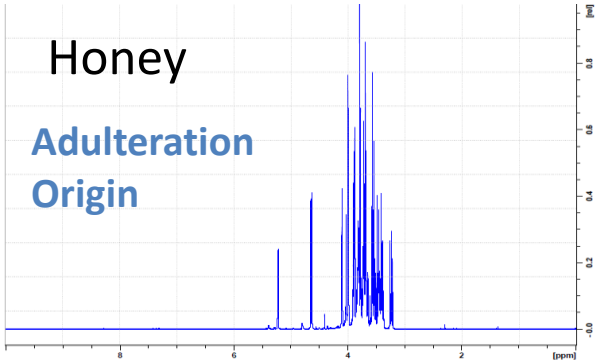
Coffee

Arabica v. Robusta
Other varieties
Adulteration



Wine

Grape variety
Adulteration



Honey

Adulteration
Origin

And more…

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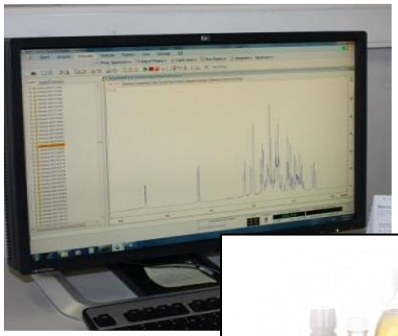
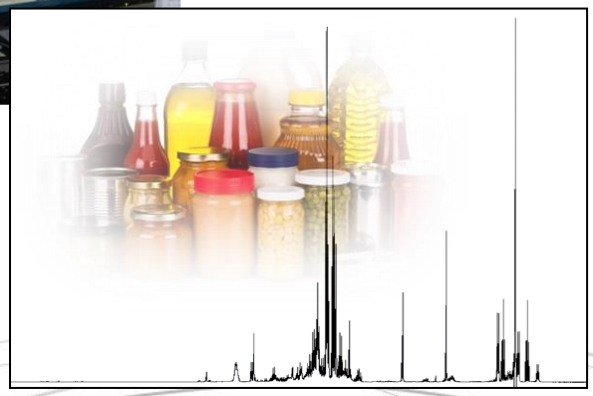
The challenges to the standardisation of non-targeted 1H NMR

Sample preparation: differences at this stage can lead to different NMR spectra for the same sample

^{1}H NMR measurement: a clear protocol is required (field strength, pulse type, acquisition time, etc.) to produce repeatable/reproducible data



Data processing and analysis: different statistical approaches can lead to different evaluations



Interpretation of product authenticity: relies on the existence of a comprehensive database

The challenges to the standardisation of non-targeted 1H NMR

What can be done to ensure standardized sample preparation and ^{1}H NMR measurement ?

- SOP preparation
- SOP acquisition
- Quality criteria

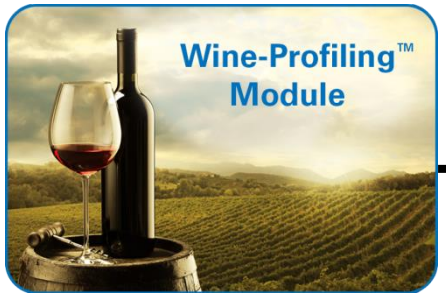
Need:

- Include internal quality control measures
- Carry out regular comparison of NMR instruments (internally and in peer to peer comparison
- Validation, qualification

Collaborative analytical tools: FoodScreener™ - Platform-Concept

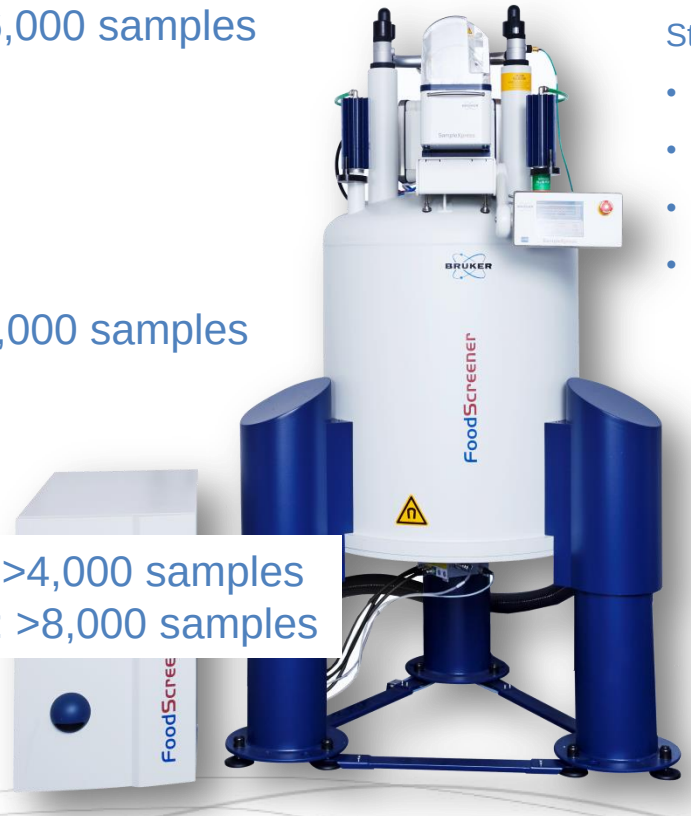
Large databases for widely spread single-ingredient commodities

>16,000 samples



>19,000 samples

Now: >4,000 samples
Soon: >8,000 samples



Standardized Platform FoodScreener™

- Quantification
- Classification models
- Verification models
- Non-targeted

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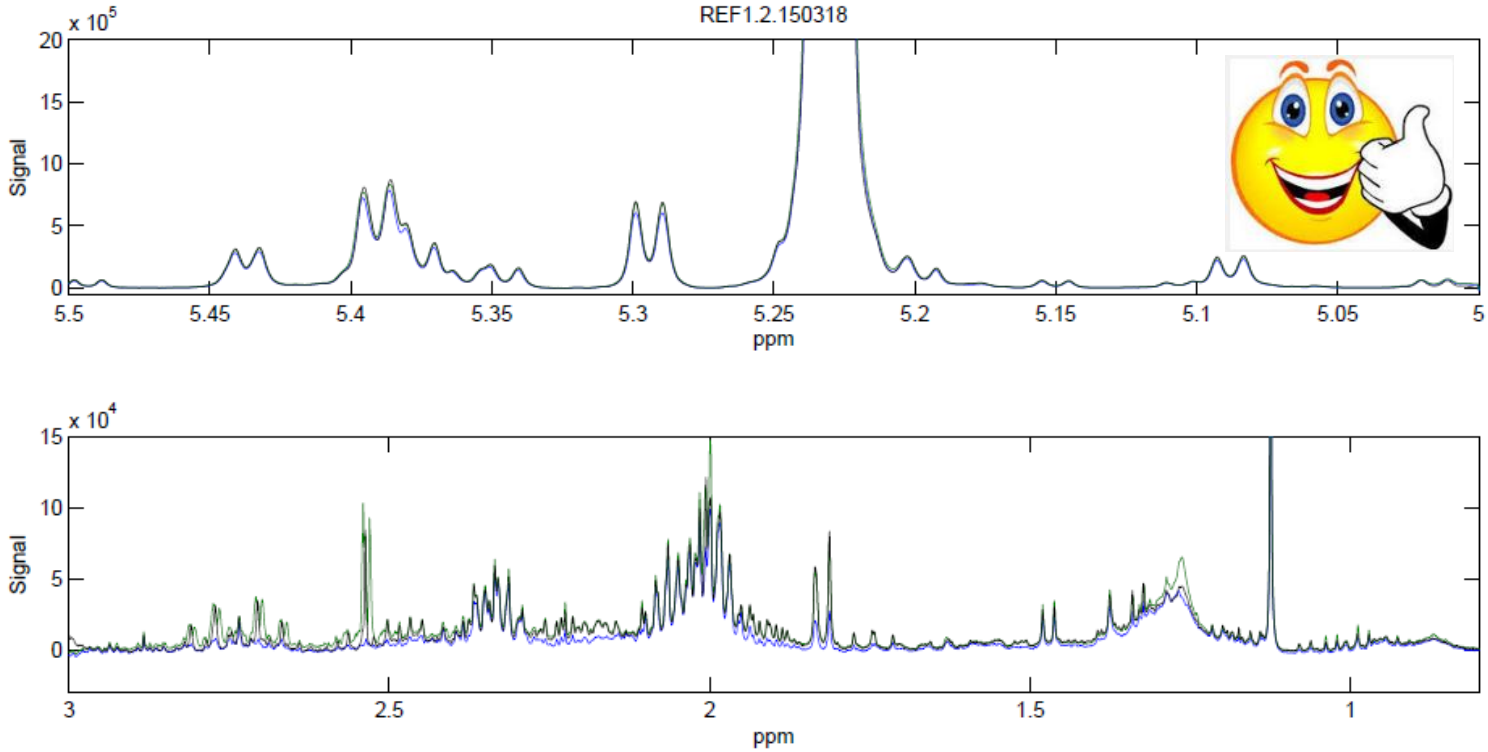
Internal Reference Material, two per session
Daily Quality Control

QC1	EX1	EX2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	QC2

Black => QC of the day

Blue and Green => upper and lower limits based on 3 weeks characterisation (more than 20 experiments)



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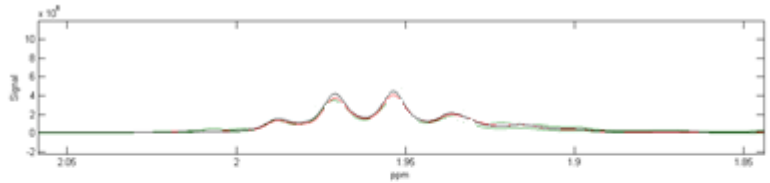
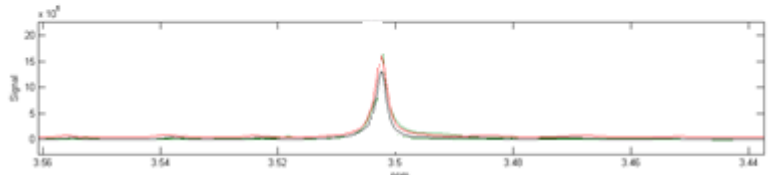
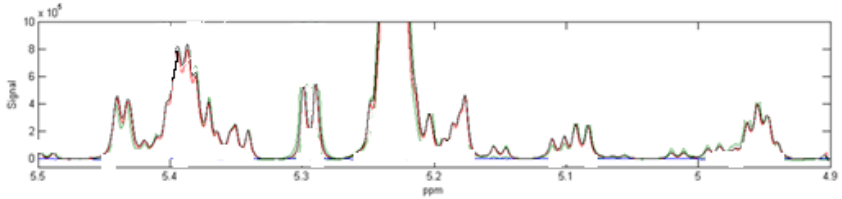
Comparison between our 2 instruments -Qualification



coffee



Red-> instrument A
Black->instrument B



honey

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Inter-laboratory process validation: Peer to Peer comparison Bruker - Eurofins

Lab Comparison

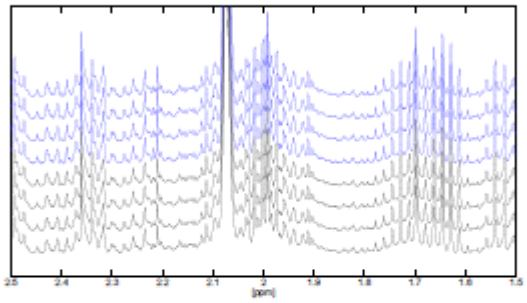
Methods: Wine-Profiling

Lab 1: Bruker BioSpin GmbH, Rheinstette
Lab 2: Eurofins, Nantes
Date: March 2013

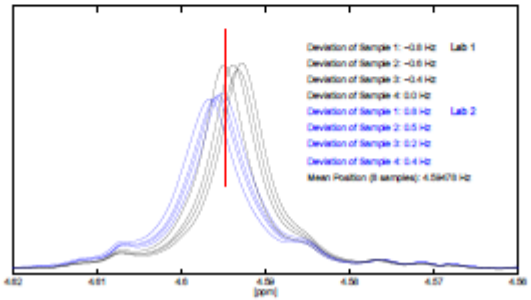
1.1 White Wine

1.1.1 Spectroscopical View

Following figure shows the spectroscopical comparison of the white wine samples prepared in both labs (4 samples in each lab, phase 3). There is no evidence for a significant difference between the preparation and measurement of both labs besides the normal achievable variation.



The signals of tartaric acid are all between 4.5820 ppm and 4.6120 ppm which indicates an appropriate sample preparation (including pH-adjustment, refer to following figure). The actual deviation between both labs coincides with the known achievable variation.



1.1.2 Quantitative Comparison

Following table shows the comparison of some quantified parameters of the white wine samples which were prepared in both labs (4 samples in each lab, phase 3).

Compound	$\bar{x}_1$ Lab1	$\bar{x}_2$ Lab2	rel. comparison [%]	rel. std. Lab2 [%]
2,3-butandiol	382	385	100.6	5.9
3-methyl-butanol	213	205	96.1	0.7
acetic acid	355	343	96.8	1.4
alanine	27	28	101.7	5.0
ethanol	93944	95557	101.7	0.6
fructose	2673	2660	99.5	1.9
glucose	762	725	95.1	12.6
glycerol	4952	4912	99.2	1.3
lactic acid	294	295	100.4	8.2
malic acid	1919	1860	96.9	1.5
succinic acid	617	594	96.3	1.8

Result:

There is no significant deviation between Lab1 and Lab2 (mean values). The standard deviation of Lab2 is for high-concentrated compounds less than 2% (e.g. ethanol, malic acid) which indicates an appropriate sample preparation.

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Periodic comparaison : wine, juice, honey

Eurofins

Sample code Nr.: 370-2016-10134150
Variety: Pinot Gris
Country: France
Region: Elsass
Vintage:
Type of Wine: white
Measuring Date: 23-Jul-2016 01:53:31
Reporting Date: 25-Jul-2016 09:28:32, Version 3.0.2, 8 pages

Results Summary

Type of Analysis	Analysis ID	Result	Status
Classification Analysis			
White Wine Variety	WI-1104-01/0681	In-Model	●
Targeted Analysis			
Quantification	WI-Q/1001	–	●
Comparison with NMR Reference Database	WI-QC/0707	–	●
Untargeted Verification Analysis			
Univariate Verification	WI-2002-02/705	In-Model	●
Multivariate Verification	WI-2002-02/705	In-Model	●
Wine Content Analysis	WI-4002-01/706	In-Model	●

Bruker

Sample ID: 4150-LAB-WI-30062016

Additional Sample Information
Customer: EUROFINS
Variety: Pinot Gris
Country: France
Region: Elsass
Vintage: 2014
Type of Wine: white
Measuring Date: 04-Jul-2016 17:56:21
Reporting Date: 12-Jul-2016 18:11:34, Version 3.0.2, 9 pages
Approval: **approved by Monika Moertter on 12-Jul-2016 18:10:00**

Results Summary

Type of Analysis	Analysis ID	Result	Status
Classification Analysis			
White Wine Variety	WI-1104-01/0681	In-Model	●
White Wine Vintage	WI-1190-01/1001	In-Model	●
Targeted Analysis			
Quantification	WI-Q/1001	–	●
Comparison with NMR Reference Database	WI-QC/0707	–	●
Untargeted Verification Analysis			
Univariate Verification	WI-2002-02/705	In-Model	●
Multivariate Verification	WI-2002-02/705	In-Model	●
Wine			

Untargeted Verification Analysis

Applied Model: Pinot Blanc/Gris

Univariate Verification

Result: No deviation was detected in univariate verification (In-Model).

Multivariate Verification

Result: No deviation was detected in multivariate verification (In-Model).

Wine Content Analysis

Result: Based on the comparison with the reference database, there is no indication for an addition of water.

Untargeted Verification Analysis

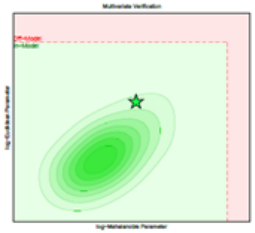
Applied Model: Pinot Blanc/Gris

Univariate Verification

Result: No deviation was detected in univariate verification (In-Model).

Multivariate Verification

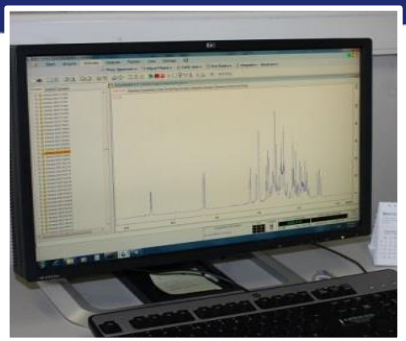
Result: No deviation was detected in multivariate verification (In-Model).



Wine Content Analysis

Result: Based on the comparison with the reference database, there is no indication for an addition of water.

The challenges to the standardisation of non-targeted 1H NMR



Data processing and analysis

- What are the effects of processing and how can discrepancies be overcome ?

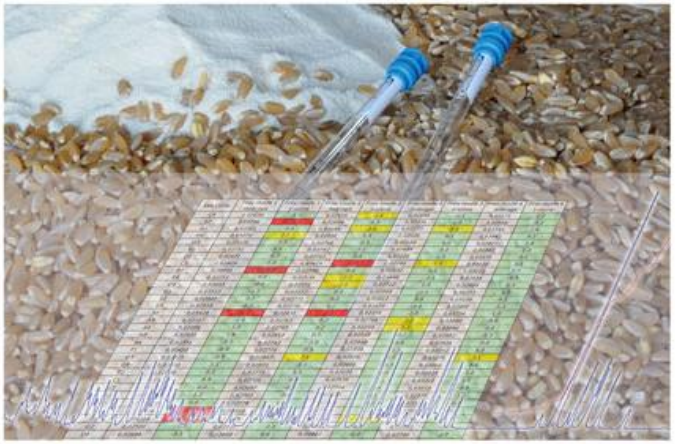
=>automatic process is the safest solution !
(Matlab routines, FoodScreeneer...)

Collaborative study Example: Wheat : organised by a PT organisator (only quantification)

NMR INTERLABORATORY COMPARISONS

INNOVATIVE SOLUTIONS

Validation of NMR fingerprinting methods: effects of processing on measure reproducibility and laboratory performance assessment



Innovative Solutions S.r.l., zona H 150/B, 70015 Noci (BA), Italy
Contact person: Prof. Vito Gallo
Phone: +39 0805963607
Email: direzione@innovative-solutions.it

Figure 1. Typical 1D ¹H-NOESY spectrum for the present ILC

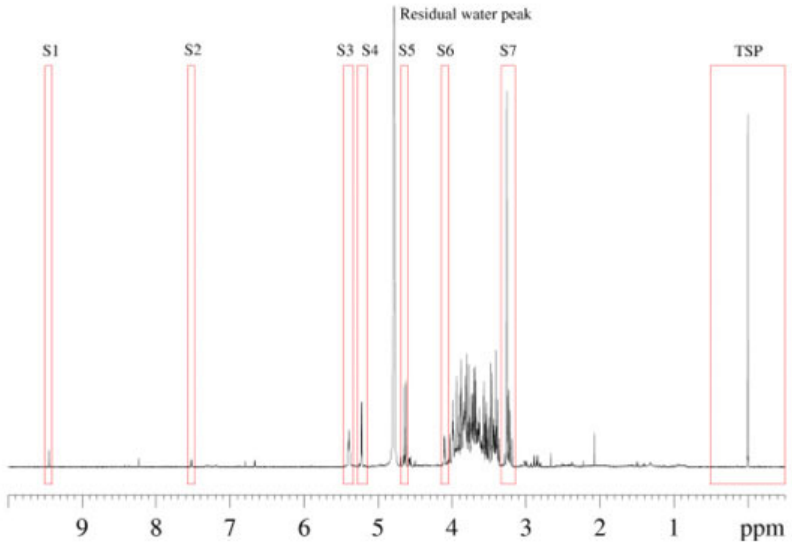


Table 2

Registered Participants	**37**
Available NMR spectrometers	46
Delivered set of samples	46
Spectrometers producing results	39
Magnetic Field (Larmor frequency)	
9.4 T (400 MHz)	16
11.7 T (500 MHz)	7
14.1 T (600 MHz)	14
16.4 T (700 MHz)	2
Spectrometer manufacturers	
Bruker	35
Varian	4

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IS NMR ILC 001_2014

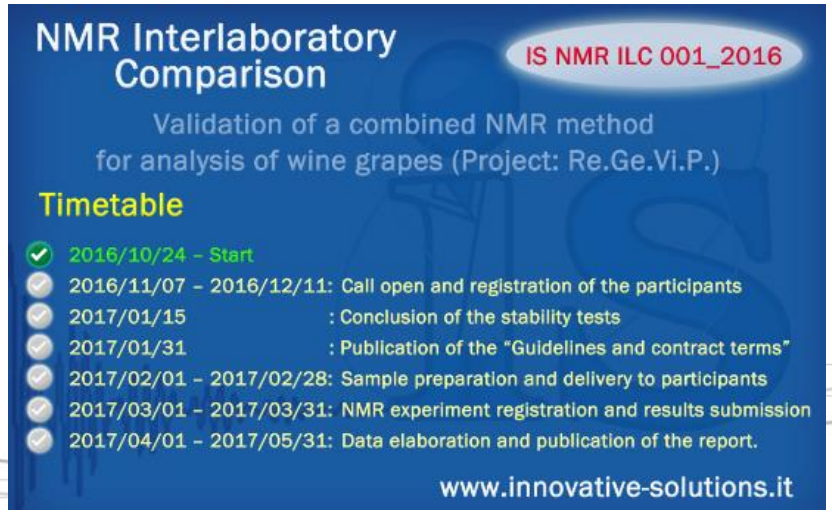
Table 3

Session	Number of operators	Processing procedure	Software	Integration mode*
ILC1[a]	many	phase and baseline correction according to operator expertise	no limitation	no limitation
ILC2	one	manual phase correction and automatic baseline correction	TOPSPIN & AMIX	integral
ILC3	one (different from session ILC2)	manual phase correction and automatic baseline correction	Mnova	sum
ILC4	one (the same as in session ILC3)	manual phase correction and automatic baseline correction	Mnova	peak

*Integration method is named differently according to the different software.

It is important to point out that laboratory performance assessment is strongly dependent on the operator. It was found that laboratories obtaining unacceptable |z-scores| (>10) in the first elaboration, ILC1, gained better results in the new elaborations ILC2-4 carried out by a single operator and in many cases their performance were satisfactory. No substantial effects of the software and of the integration procedure were found.

Integral ratio	I_{S1}/I_{TSP}							
Tube	B							
Lab Code	ILC1 Average	ILC1 Z-score	ILC2 Average	ILC2 Z-score	ILC3 Average	ILC3 Z-score	ILC4 Average	ILC4 Z-score
C5	0.02420	-1.0	0.02516	-2.4	0.02498	-0.1	0.03174	1.3
E3	2.63706	1228.9	0.02604	-1.5	0.02328	-1.6	0.02986	-0.3
A1	0.02383	-1.1	0.02493	-2.6	0.02172	-3.0	0.03194	1.4
H4	0.03070	2.1	0.02928	1.9	0.02526	0.1	0.03042	0.2
G5	0.02520	-0.5	0.02714	-0.3	0.02580	0.6	0.02822	-1.6
B1	0.02751	0.6	0.02742	-0.1	0.02498	-0.1	0.02862	-1.3
D1	0.02471	-0.7	0.02711	-0.4	0.02398	-1.0	0.02970	-0.4
C3	0.02409	-1.0	0.02415	-3.4	0.02246	-2.3	0.03036	0.1
B2	2.70651	1261.6	0.02706	-0.4	0.02532	0.1	0.03102	0.7
F4	0.02753	0.6	0.02535	-2.2	0.02498	-0.1	0.02834	-1.5
D3	0.02493	-0.6	0.02551	-2.0	0.02722	1.8	0.02956	-0.5
G1	0.02312	-1.5	0.02772	0.3	0.02504	-0.1	0.03252	1.9
E1	0.02680	0.3	0.02771	0.3	0.02612	0.8	0.03036	0.1
E5	0.02558	-0.3	0.02770	0.2	0.02470	-0.4	0.02868	-1.2
B3	0.05428	13.2	0.02455	-3.0	0.02394	-1.0	0.03254	1.9
A5	0.02207	-2.0	0.02765	0.2	0.02764	2.2	0.03090	0.6
A4	0.02301	-1.5	0.02765	0.2	0.02764	2.2	0.03090	0.6
H2	0.02924	1.4	0.02813	0.7	0.02538	0.2	0.02982	-0.3
B4	0.02551	-0.3	0.02742	-0.0	0.02448	-0.6	0.02924	-0.8
F1	0.02458	-0.8	0.02776	0.3	0.02338	-1.5	0.03062	0.4



In line with the results reported in the previous volume, the new data elaboration indicate that the NMR experiment (1D ^{1}H NOESY) proposed for the fingerprinting of wheat and flour aqueous extracts is a robust experiment. In fact, the majority of the participants produced NMR spectra that can be considered "statistically equivalent". Thus, the main goal of this inter-laboratory comparison, the validation of the 1D ^{1}H NOESY experiment, was achieved.

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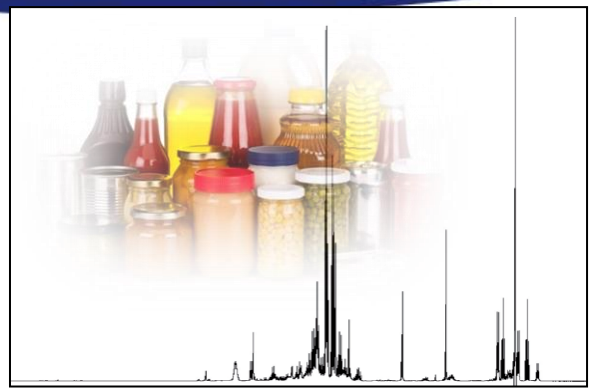
The challenges to the standardisation of non-targeted 1H NMR



How to avoid errors in the interpretation of product authenticity

=>a unique validated database is the key!

Validation Files
- Classification Models -

- Validation by Monte-Carlo/Cross-Validation
- Analysis of Confounders
- Wrong prediction rate < 3%

3.2.1 PLIMIT = 0.01

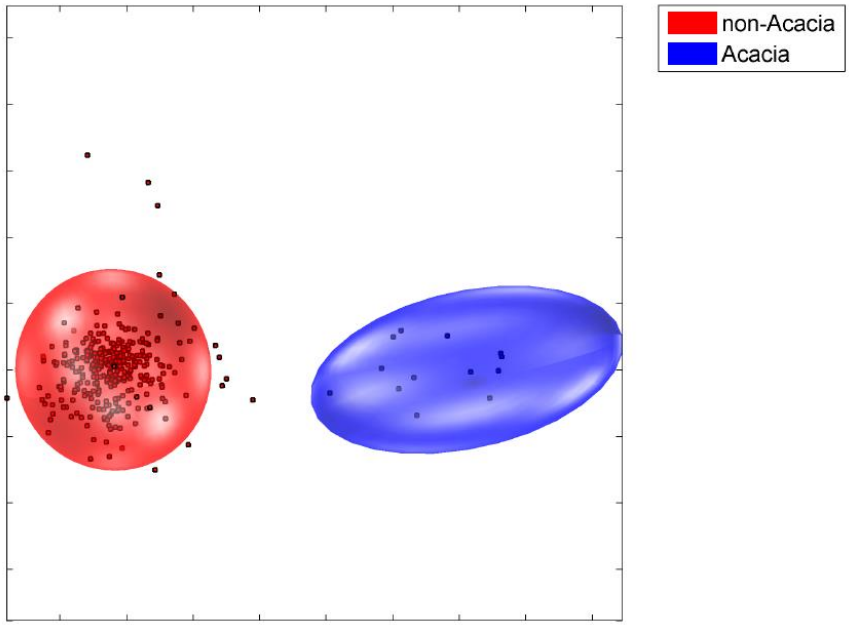
	1	2	Amb.OK	None	Wrong
1: non-Acacia	96.2	2.3	0.0	1.5	2.3
2: Acacia		97.2	0.0	2.8	

	1	2	Double	Triple
1: non-Acacia			0.0	0.0
2: Acacia			0.0	0.0

Correct Prediction Rate Unique: 96.2%
Correct Prediction Rate Ambiguous: 0.0%
Correct Prediction Rate Total: 96.2%
False Prediction Rate: 2.2%
Prediction Rate 'None': 1.6%

Overview of classes

Class	Samples	Percentage
non-Acacia	2753	96.3
Acacia	107	3.7
Total	2860	100.0



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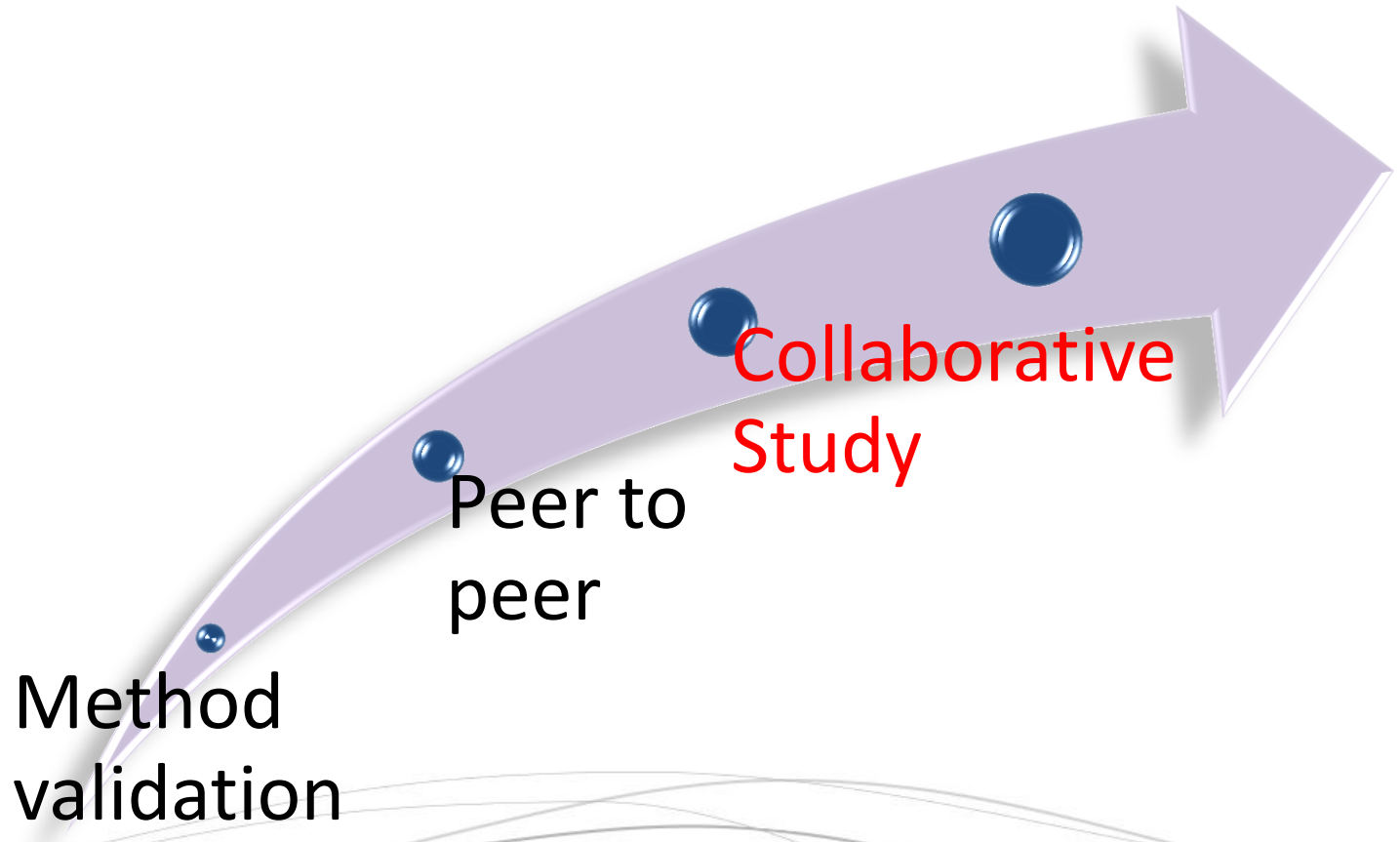
Larger Collaborative studies already made



1-Juice : In the frame of BIPEA PTS

SGF Interlaboratory Comparison 2015

Apple juice from concentrate

5 instruments
routine conditions
(=PTS)

4. Results from SGF-Profiling™

In our 2015 Inter-laboratory Comparison all cooperation laboratories using the SGF-Profiling™ in their routine work participated with this proton NMR technique, as it was done in 2014.

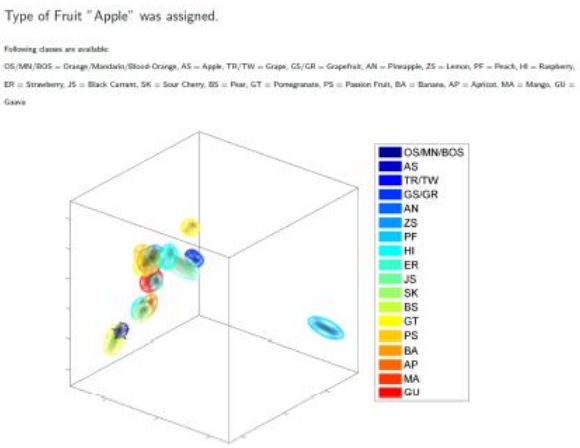


Figure 1: Assignation of sample to Apple juice

The results provided from these laboratories are quite impressive since all of them identified the samples as apple juice and as 100% juice from concentrate.

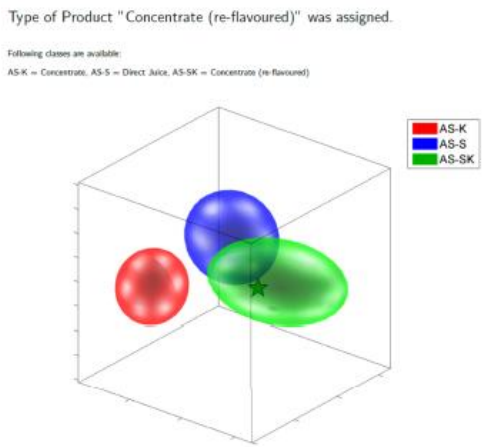


Figure 2: Assignation of product type: from concentrate

All the participating laboratories reported that the sample fits in the multivariate verification model:

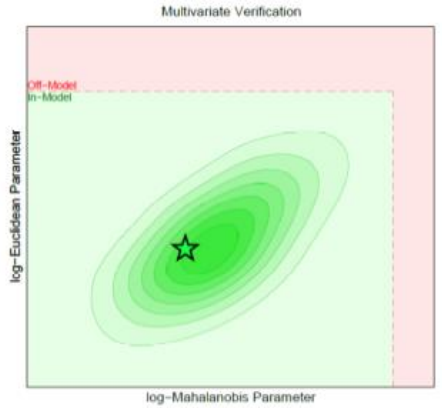


Figure 3: Sample fitting in the multivariate verification model

As for the analysed parameters all the laboratories gave similar values, and moreover, the obtained values are very much in line with the target values. In Table 4, the values obtained by the NMR can be seen.

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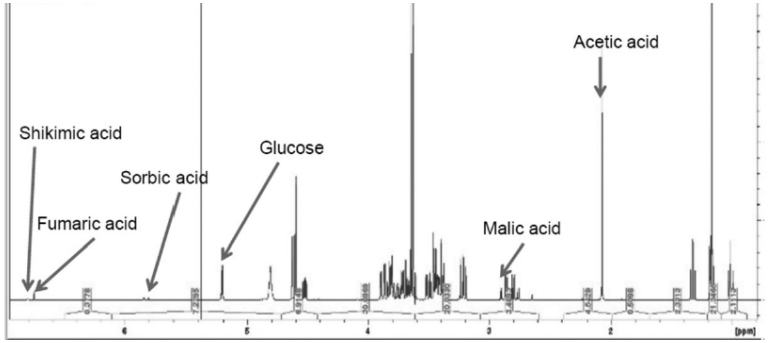
2-Wine : organised by Dr. Ristow

FOOD COMPOSITION AND ADDITIVES

Quantitation of Compounds in Wine Using ^{1}H NMR Spectroscopy: Description of the Method and Collaborative Study



Anlage 1: Verzeichnis der Teilnehmer

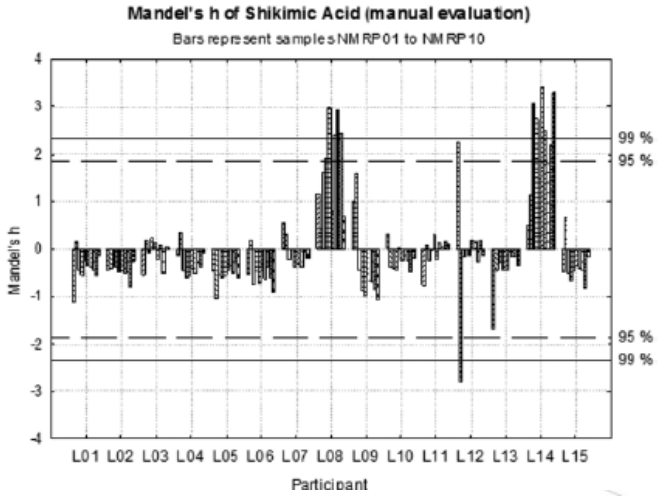
ALNuMed GmbH	D-95448	Bayreuth
BAM Bundesanstalt für Materialforschung	D-12489	Berlin
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	D-97082	Würzburg
BfR Bundesinstitut für Risikobewertung	D-10589	Berlin
Bruker BioSpin GmbH Rheinstetten	D-76287	Rheinstetten
Bruker Italia	I-20158	Milano
c h e l a b Hemmingen	D-30966	Hemmingen (Han.)
Chemisches und Veterinäruntersuchungsamt Karlsruhe	D-76187	Karlsruhe
Eurofins Analytics - Nantes	F-44323	Nantes Cedex 3
Eurofins Analytik GmbH - Hamburg	D-21079	Hamburg
Hochschule Geisenheim University	D-65366	Geisenheim
Institut Heidger	D-54518	Osann-Monzel
LGC Teddington	GB-TW110LY	Teddington Middlesex
Quality Services International GmbH	D-28199	Bremen
WINESPIN ANALYTICS GmbH & Co.KG	D-55459	Aspisheim

15 instruments

Manual integration

Prüfgut-Kode	Externer Kode	Jahrgang	Beschreibung
NMRP01		ohne	Modellwein
NMRP02	DWA 1050807	ohne	Standardlösung zur Weinanalytik
NMRP03		2011/2012	Merlot di Venetia DOC, dotiert
NMRP04	FT14P02	2011	Dornfelder und Spätburgunder, Rheinhessen
NMRP05	DWA 1061305	ohne	Standardlösung zur Weinanalytik
NMRP06	FT14P04	ohne	Französischer Rotwein „Medinet", halbtrocken
NMRP07	FT13P02	2010	Scheurebe, Pfalz (säurereich)
NMRP08	FT14P05	2013	Riesling, Pfalz
NMRP09		2011	Chardonnay, Central Ranges, Australien, dotiert
NMRP10		2013	Cabernet Sauvignon, Central Valley, Chile, dotiert

Analyte	Mean recovery, %[a]	Variation, %	Methods of determination[b]
Glucose	100.0 (n = 9)	95.2–106.4	OIV MA-AS-311-02 and MA-AS-311-03 and equivalent enzymatic and HPLC methods
Malic acid	104.1 (n = 7)	91.4–124.1	OIV-MA-AS313-11 and MA-AS313-12A/12B and equivalent enzymatic and HPLC methods
Acetic acid	108.9 (n = 10)	99.9–123	Enzymatic and HPLC methods
Fumaric acid	96.8 (n = 6)	80.5–104.1	HPLC methods
Shikimic acid	105.2 (n = 10)	91.2–122.3	OIV MA-AS313-17 and equivalent HPLC methods
Sorbic acid	100.2 (n = 8)	97.9–102.5	OIV MA-AS313-20 and equivalent HPLC methods



8 instruments

Automatic integration

Part of the collaborative study included the integration of signals and data evaluation in automatic mode with WineScreener™ (Bruker BioSpin).

This indicates that the elimination of personal effects/ influences during the spectral evaluation did have a decisive influence on the reproducibility of the results, notwithstanding uniform instrumental equipment and measurement settings.

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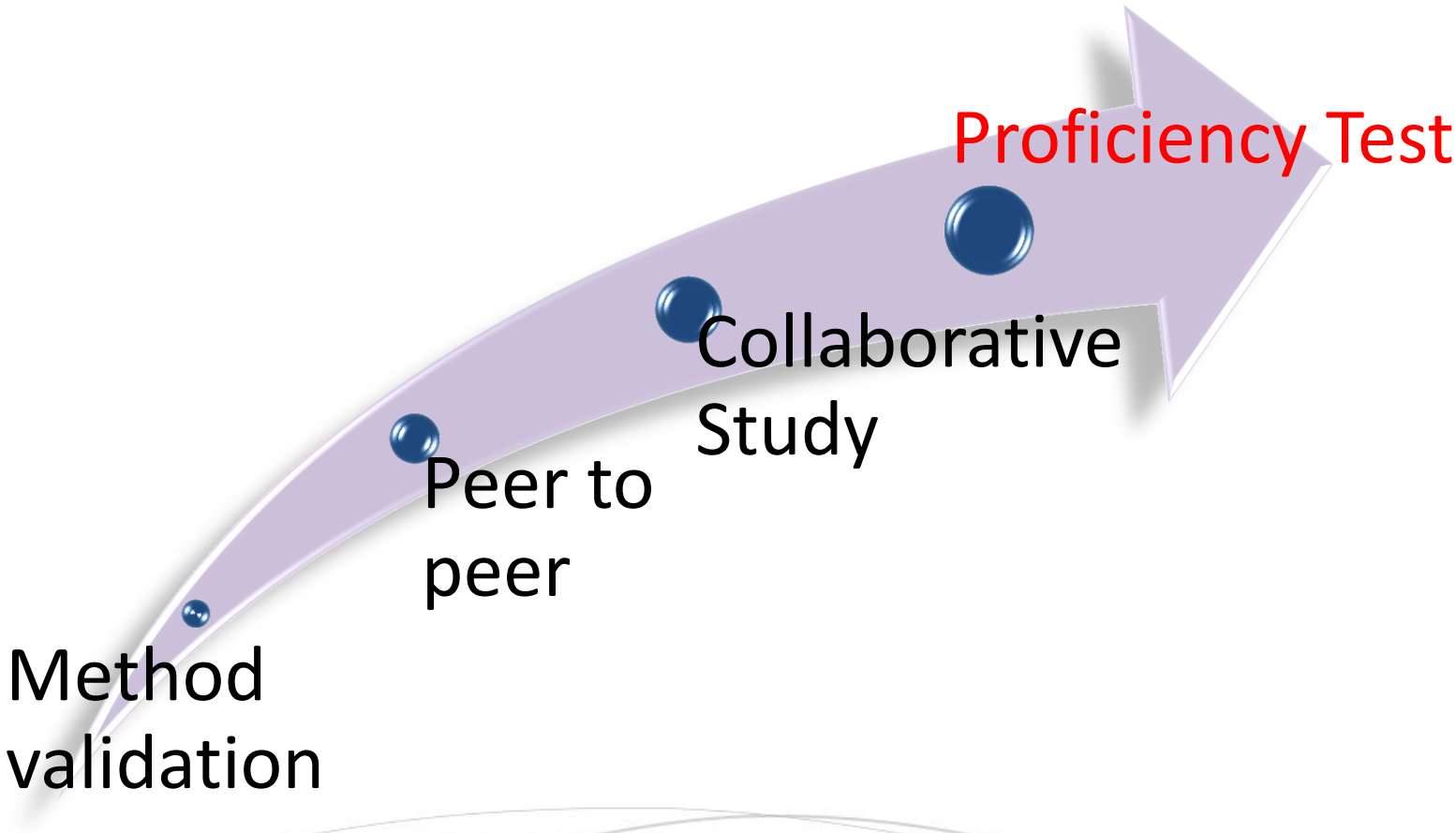
Next challenge



Our suggestion : from FIT-PTS towards Profiling-PTS

- **FIT-PTS:**

Initiated in 1994 => more than 20 years of experience !

Dedicated to Food analysis using Isotopic Techniques (IRMS, SNIFNMR)

Complies with the ISO/IUPAC/AOAC International Harmonised Protocol for Proficiency Testing of analytical laboratories

70 participants (worldwide)

Recognized by accreditation bodies

Our vision of future NMR profiling proficiency testing

- **Profiling PTS**

Initial project: matrices shared with isotopic PTS (Wine, Juice, Honey)

1 sample per trimester

Targeted : calculation of z-scores
Non-targeted: Classification scores? ….

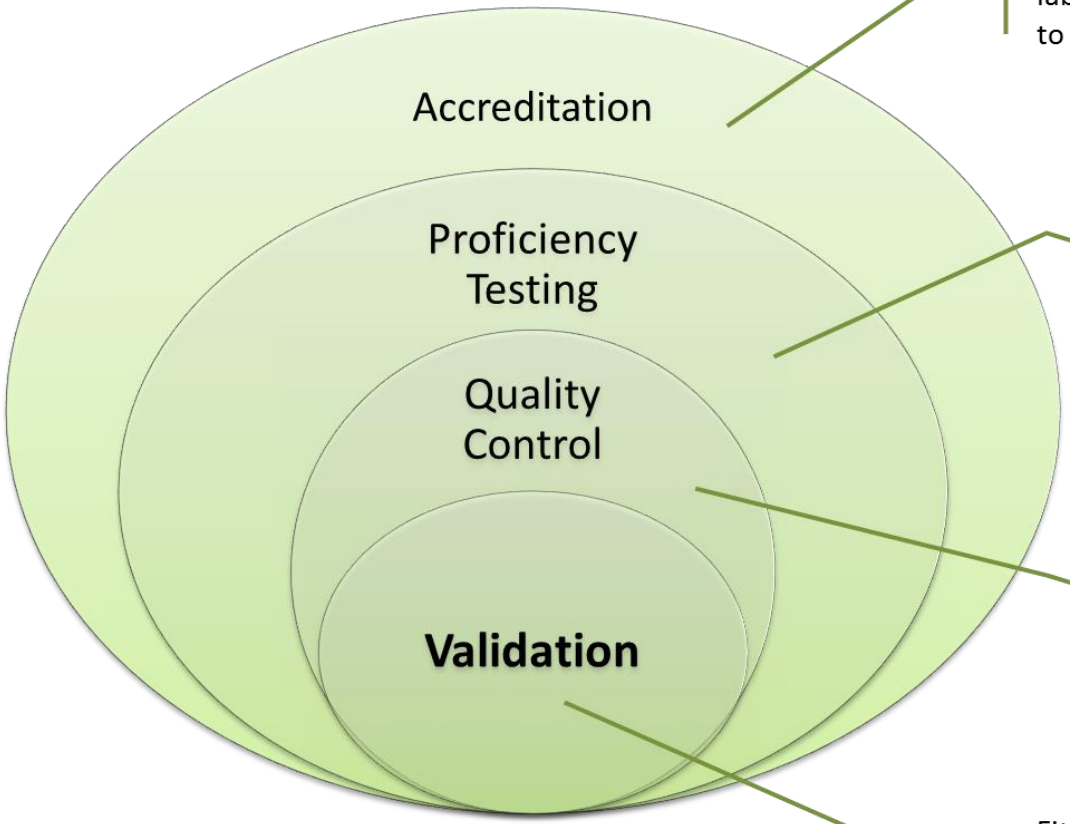
Spectra evaluation and quantifications?

A need for accreditation and commercial acceptance

First Round will be in 2017, in parallel with the FITPTS distribution,

Eurofins will take care of the organisation (preparation, parcels, results...)

Accreditation process



Formal recognition of laboratory's competence to carry out a specific test

Performance assessment of participating laboratory

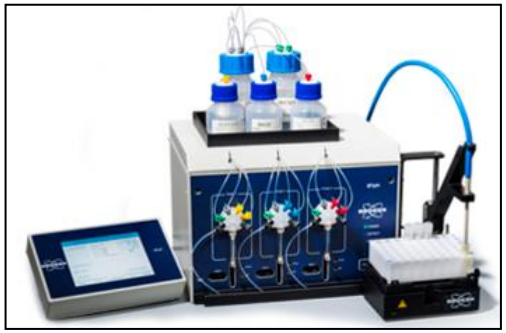
Continuous verification of analytical system to ensure consistent quality
IQC procedures: replicates, control charts, reference materials or spiked samples

Fitness for purpose of analytical system (method protocol, type of matrix, concentration range of analyte)

Source: I. Tavernier, M. de Loose, E. Van Bockstaele, Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. 2004, *Trends in Analytical Chemistry*, 23(8), 535-552.

ISO 17025: inter-laboratory accreditation

Preparation



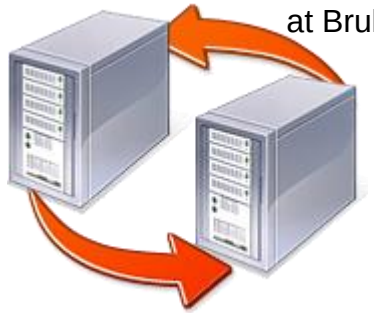
NMR-Tube →

Acquisition

encrypted data transfer →
← results/report

Analysis and Reporting

Data Analysis Server at Bruker



- standardized operating procedure (SOP)
- Criteria to have spectra validated (SOP)
- reference database and analysis routines (chemometrics, models) (SOP)

cofrac ESSAIS Accréditation n° 1-0287 Portée disponible sur www.cofrac.fr

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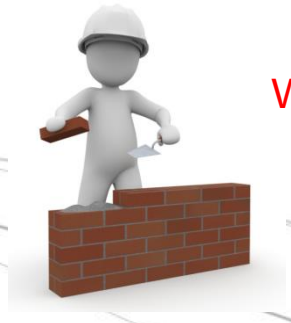
DAkkS Deutsche Akkreditierungsstelle D-PL-19229-01-00

Ok with ISO 17025
(= subcontract of the last part)

cofrac ESSAIS Accréditation n° 1-0287 Portée disponible sur www.cofrac.fr



Work in progress

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Thank you for your attention!

Roundtable Discussion after Jana's speech...

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