Non-targeted Analysis of Selected Hoffmann Toxicants in Smoke Condensate by Cryoprobe 1H NMR

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Outline

- Introduction
- Screening analytical techniques
- Nuclear Magnetic Resonance
  - Advantages and limitations
  - Whole tobacco smoke screening
  - Results
  - Next steps

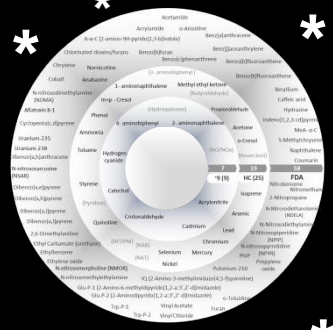
Introduction

- **Tobacco smoke – extremely complex matrix containing 6000 + substances with largely different physico-chemical properties**

  → challenging task for determination of constituents

- **Analytical strategies for measurement of constituents**
  - **Targeted analysis (established methods)**
  - **Exploratory analysis (screening techniques)**

The Lists



Analytical approaches

Established Methods

- Targeted analysis of a single compound / suites of substances of similar physico-chemical properties
- Multi step analytical procedure
- Usually fully validated
- High confidence in the results
- Often time consuming
- Labour intensive
- Limited flexibility

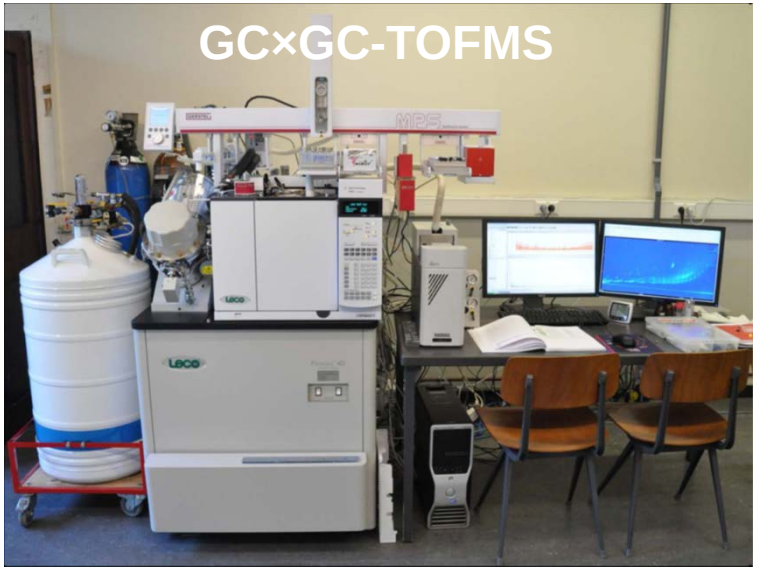
Limited scope

Screening Techniques

- Identification and semi-quantification of extended suites of target analytes
- Fingerprinting and comparison at a greater level of detail
- Only “partial” validation
- Non-targeted screening of multiple analyte groups in complex matrices
- Method development tool for confirmatory methods

Broad scope

Chromatographic screening techniques



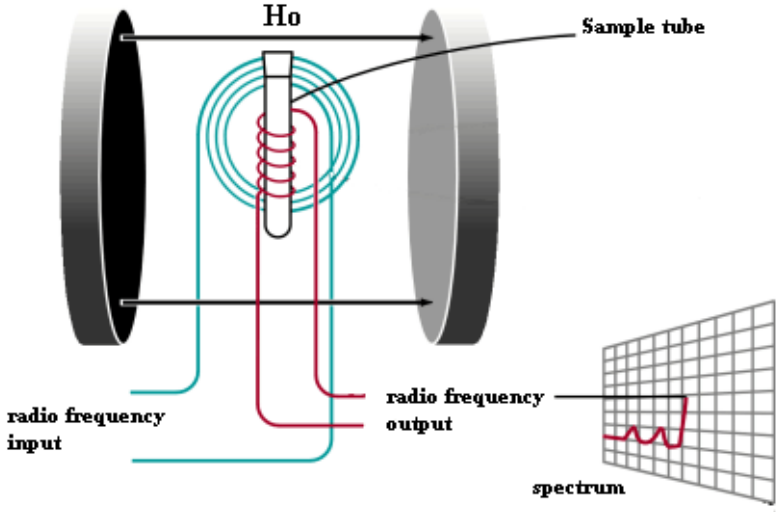
Nuclear Magnetic Spectroscopy

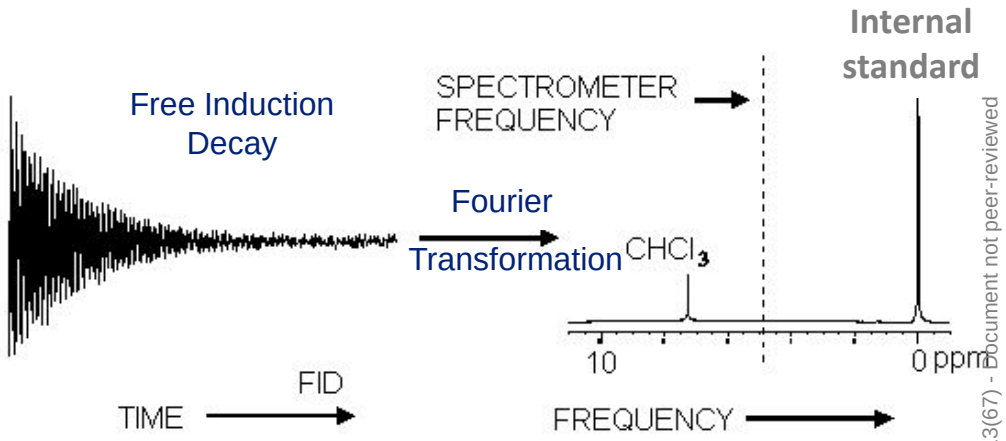
- Detailed information about molecules and their environment

- ***Principle***

Radiofrequency waves are used to excite nuclei in a magnetic field at their resonance frequency (RF)

- Four sequential steps:
  - The net alignment of the magnetic nuclear spins with an applied constant magnetic field
  - The perturbation of this alignment by employing radio frequency (RF) pulses
  - Measuring the current induced as the nuclei relax to realign with the magnetic field (Free Induction Decay)
  - Fourier transform of the time domain data to generate the frequency domain NMR





From: http://www.mhhe.com/physsci/chemistry/carey/student/olc/ch13nmr.html , http://ishigirl.tripod.com/pchem/nmr.html (accessed 21 Aug 2013)

Benefits of NMR

- Targeted and non-targeted analysis
- Robust (minimal matrix effects)
- Non destructive
- Fast answers and high confidence in chemical identity
- Structural information
- NMR library

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Fast screening approach for identification and semi-quantification of tobacco smoke constituents

Characterisation of tobacco smoke condensate

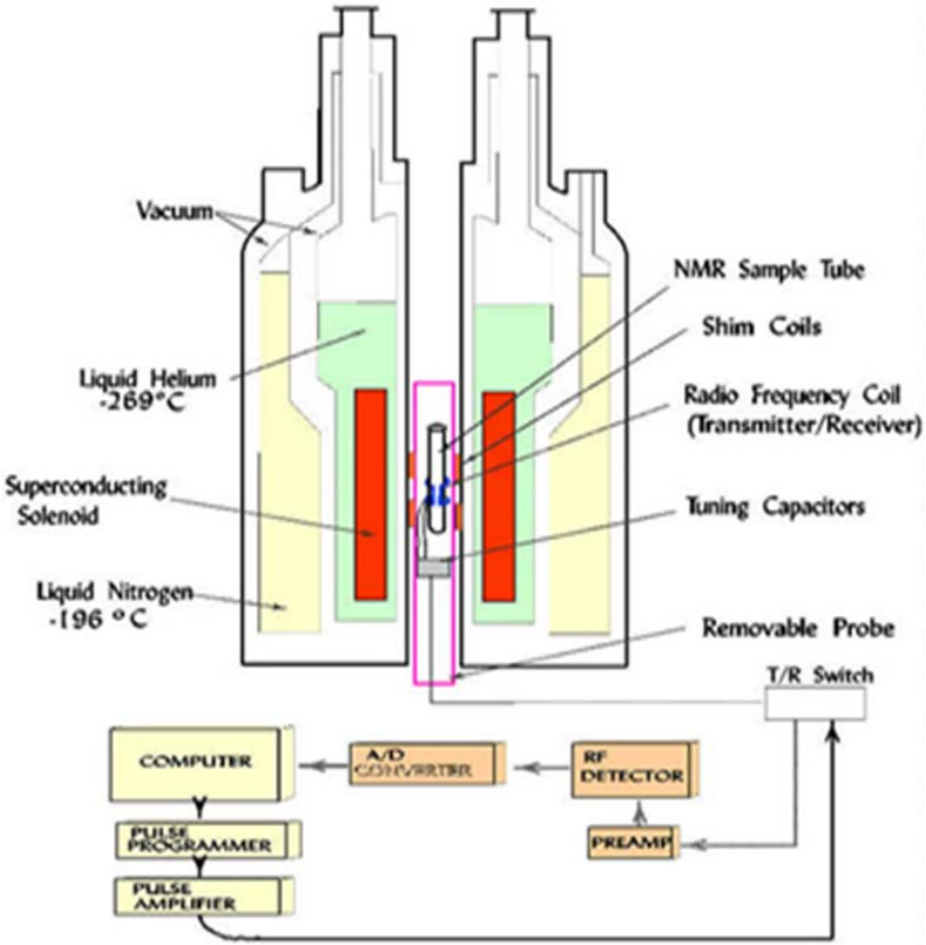
Confirmatory technique and guided method development

Compatible with industry standards sample preparation strategies

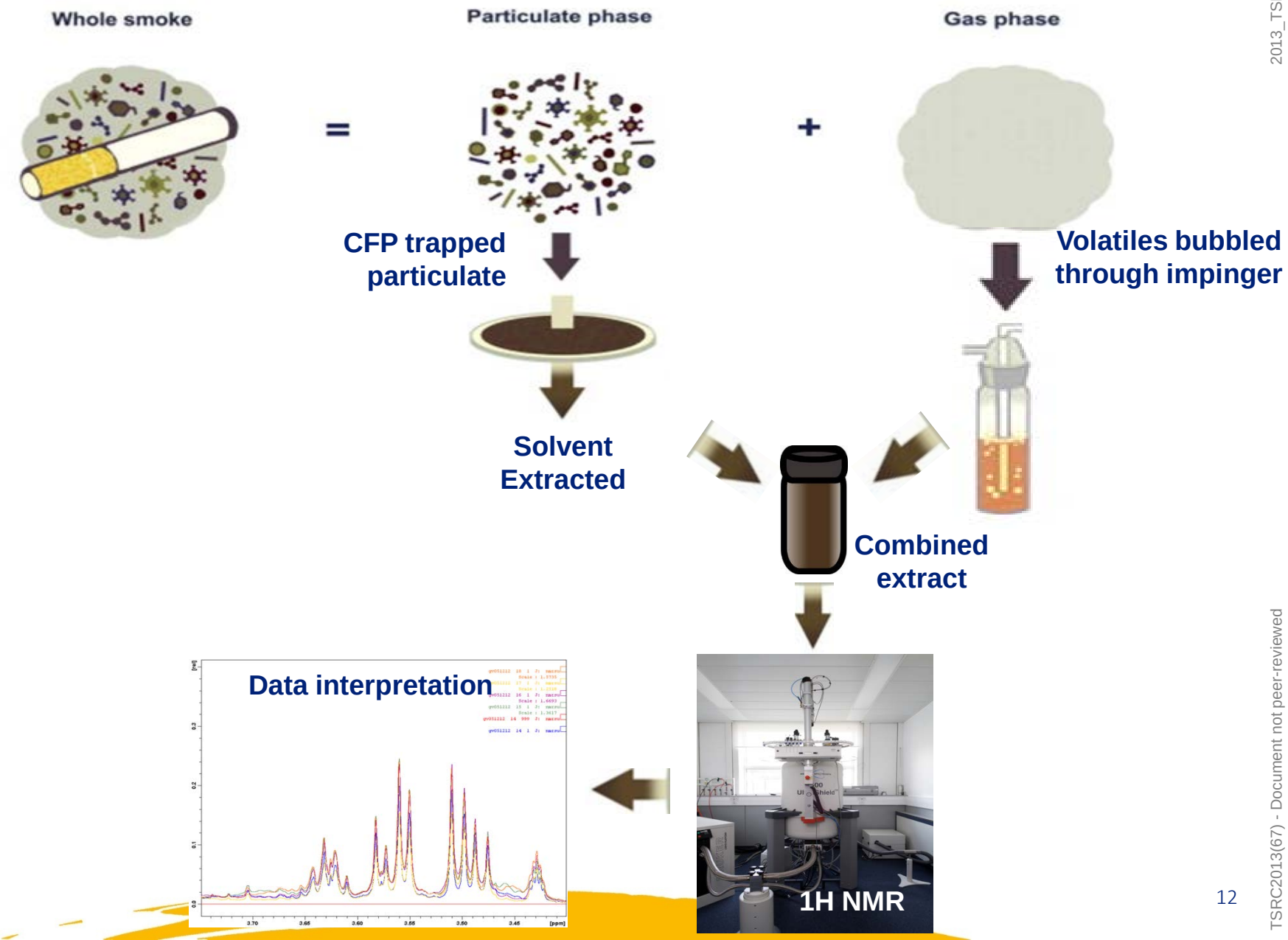
Development of NMR capability: BAT and FERA collaboration

- **Food and Environment Research Agency (FERA)**

Cryoprobe ^{1}H NMR



Sample preparation workflow



Results

Feasibility study

- **33 Hoffmann analytes representing different chemical classes excluding inorganics, metals and NFDPM**
  - **20 analytes readily detected in tobacco smoke condensate**
  - **13 analytes not detected ⇒ 12 analytes could be detected at higher concentration levels by fortifying experiments (butyraldehyde - no resolvable peaks)**
  - **NMR database**

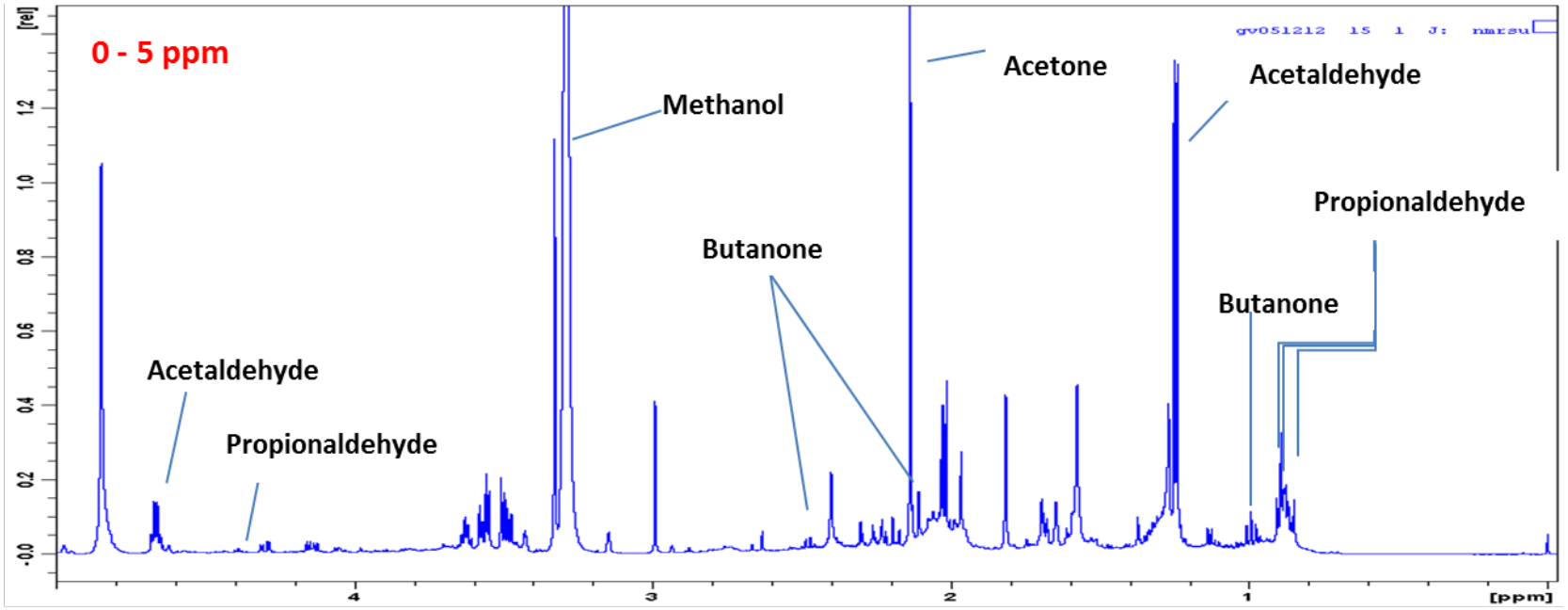
Validation

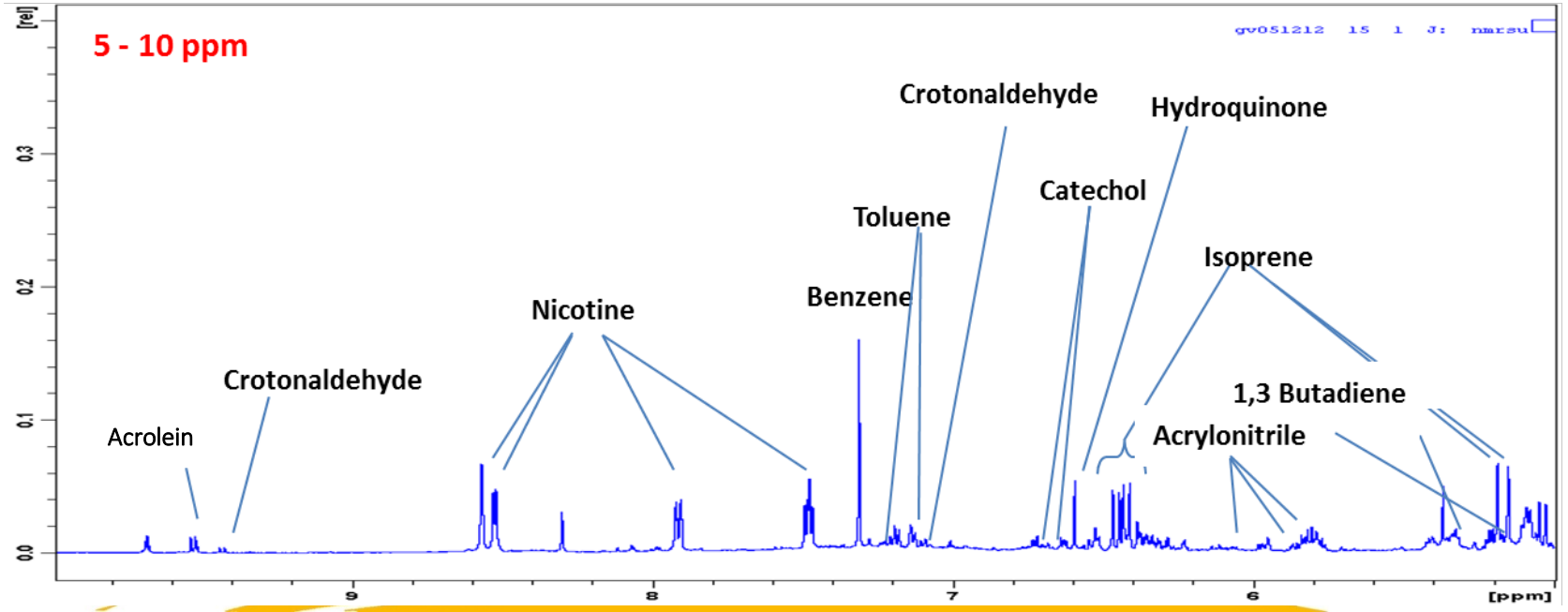
- **Validation of the methodology - overspiking experiments**
- **Verification of the robustness methodology - "blind" test samples**

Detectability of the analytes in 3R4F smoke condensate

Compound in 3R4F whole smoke condensate		
Present > LOQ. Detectable by NMR.	Detectable by NMR at higher concentration levels.	Not detectable by NMR.
Acetaldehyde	1,3-butadiene	Butyraldehyde
Isoprene	N-nitrosoanatabine	
Nicotine	N-nitrosoanabasine	
Acetone	N-nitrosonornicotine ketone	
Acrolein	Benzo(a)pyrene	
Toluene	4-aminobiphenyl	
Catechol	Resorcinol	
Hydroquinone	Quinoline	
Formaldehyde	N-nitrosonornicotine	
Acrylonitrile	1-aminonaphthalene	
Propionaldehyde	2-aminonaphthalene	
Crotonaldehyde	3-aminobiphenyl	
Butanone		
Benzene		
Pyridine		
Phenol		
Styrene		
o-Cresol (2-methylphenol)		
m-Cresol (3-methylphenol)		
p-Cresol (4-methylphenol)		

NMR profile of 3R4F (5 cigarettes smoked, ISO)





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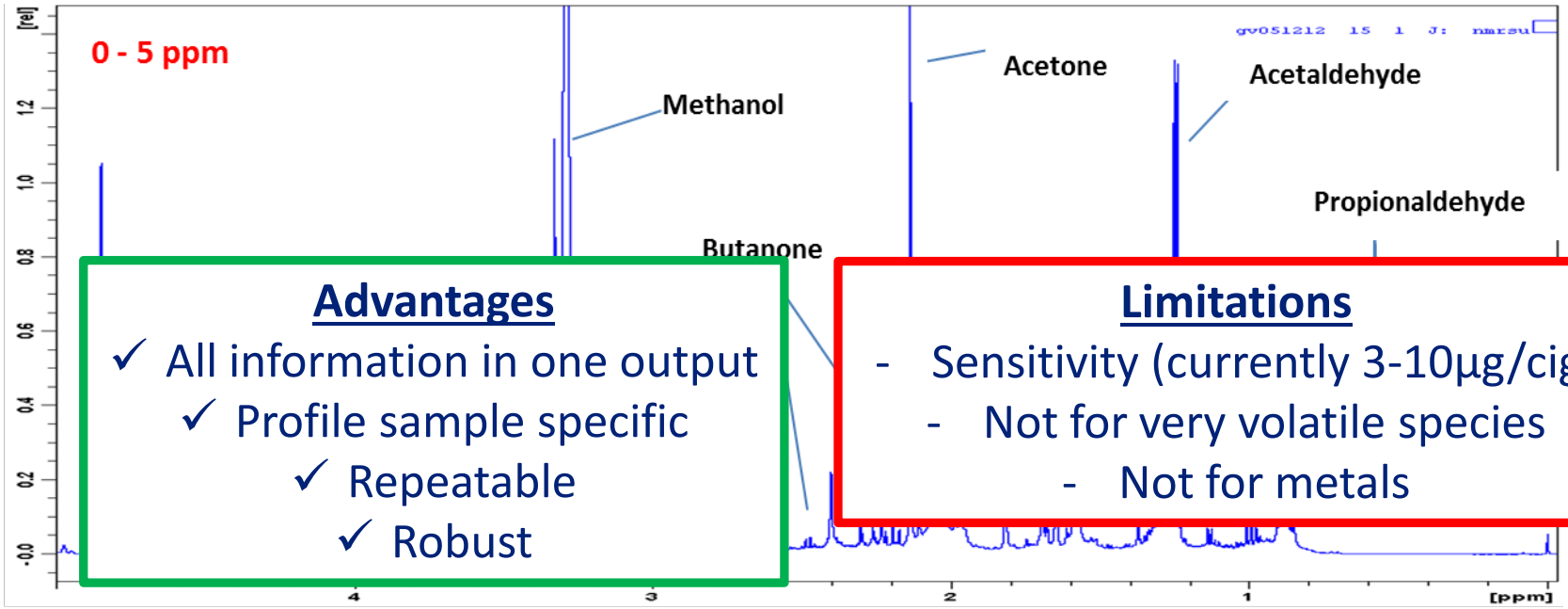
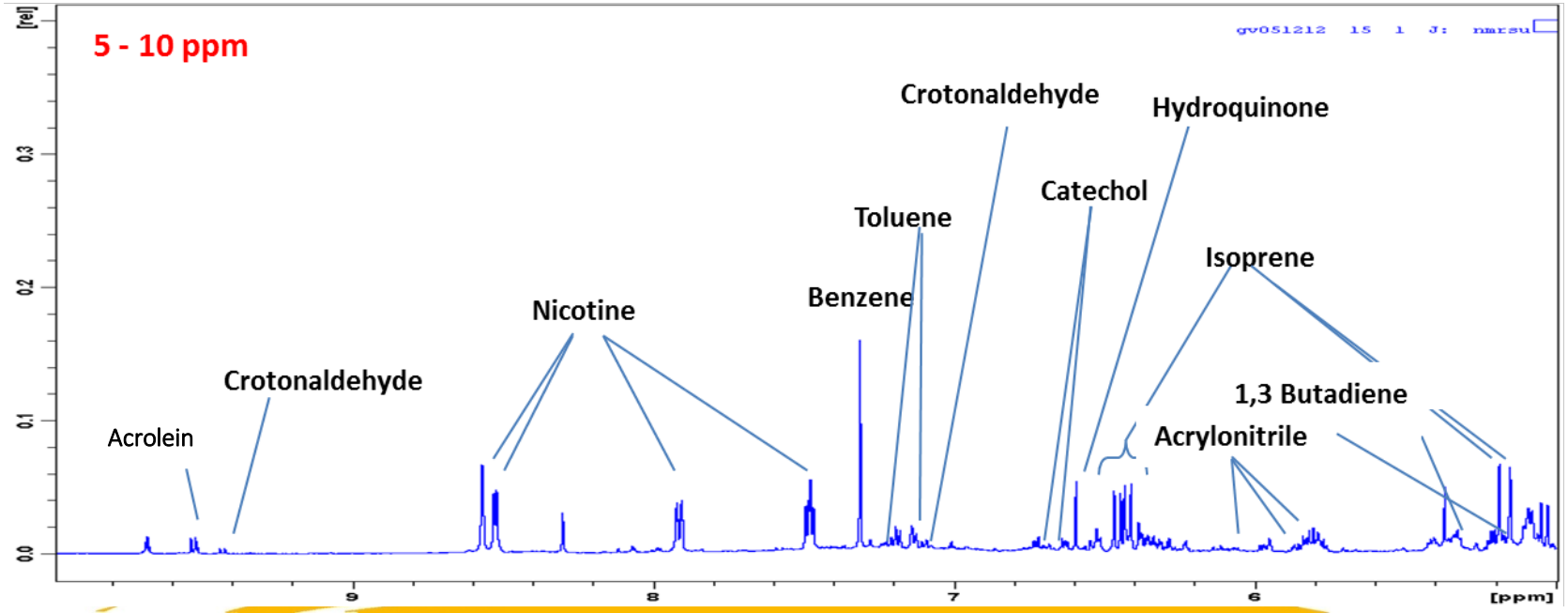


Advantages

- ✓ All information in one output
- ✓ Profile sample specific
- ✓ Repeatable
- ✓ Robust

Limitations

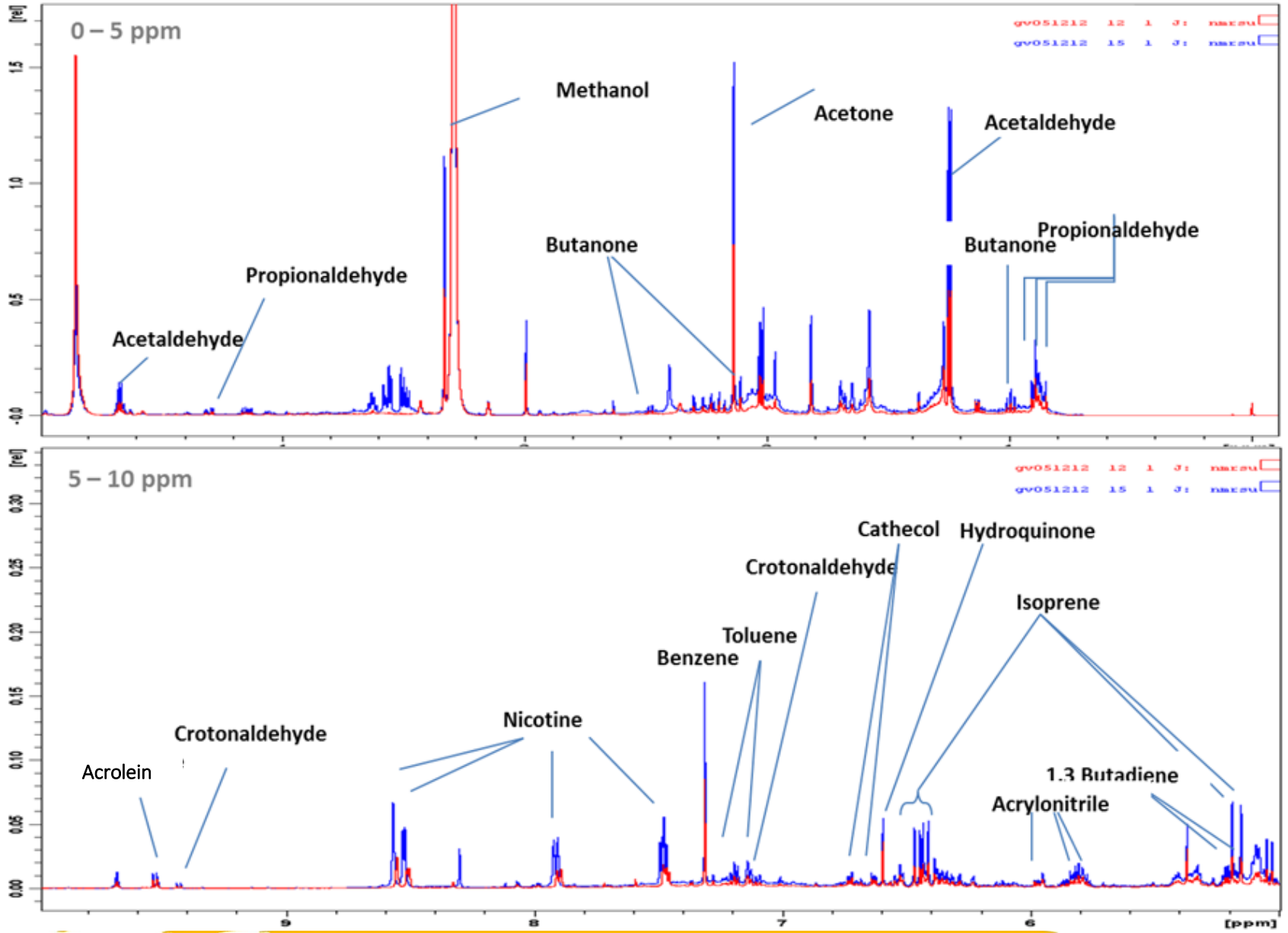
- Sensitivity (currently 3-10µg/cig)
- Not for very volatile species
- Not for metals



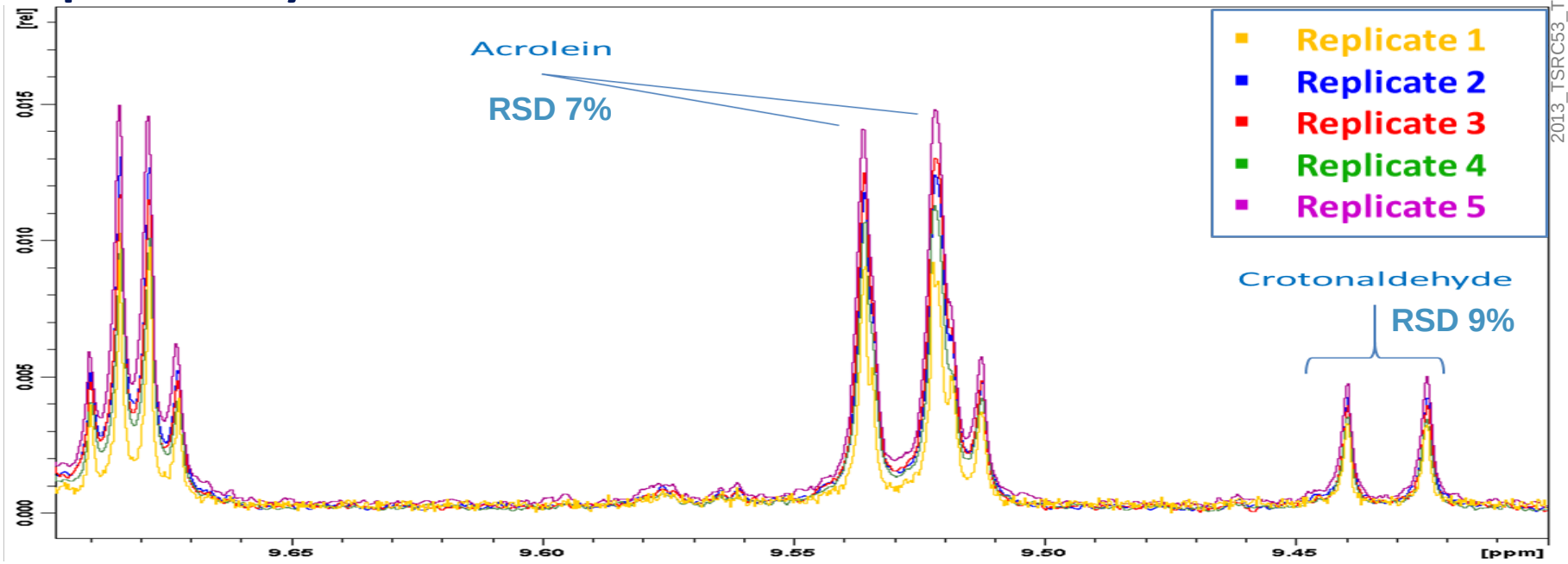
Differentiation of matrices

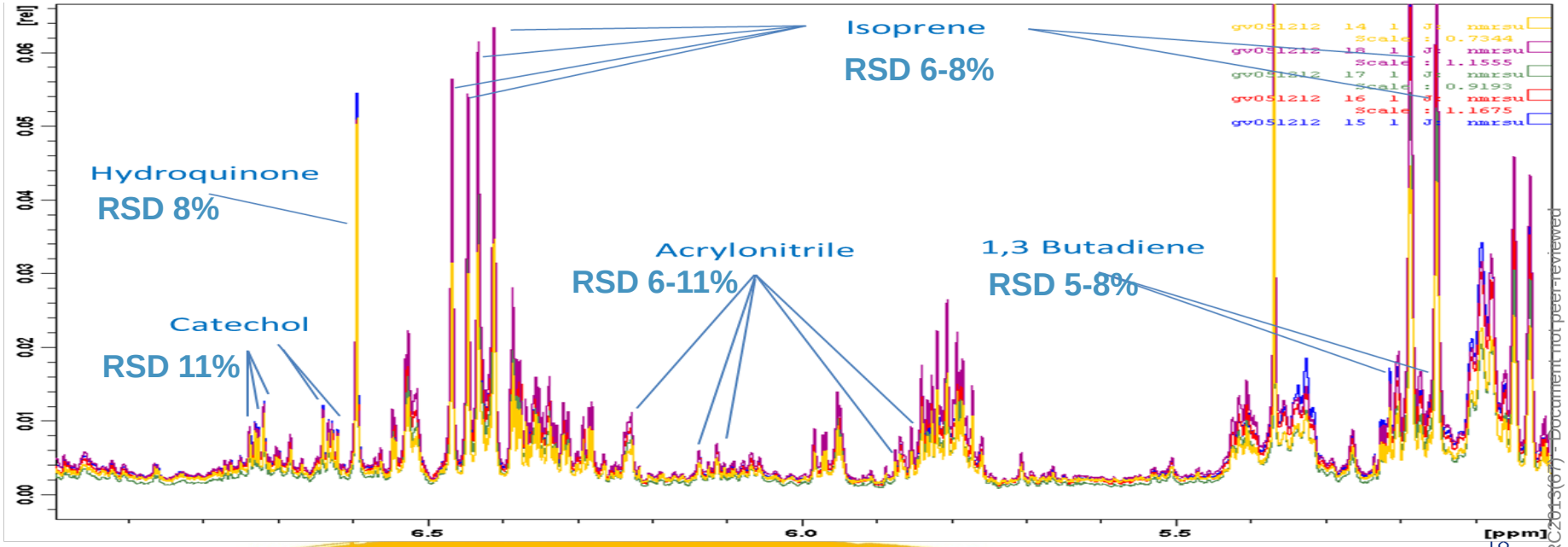
Sample 1: NFPDM ~ 9 mg/cig

Sample 2: NFPDM ~ 4 mg/cig



Repeatability





Summary

- 33 Hoffmann analytes tested
  - 20 readily detected
  - 12 analytes detectable at higher concentration
  - Butyraldehyde - no resolvable peaks
- Robust and repeatable methodology
- Sample (matrix) specific
- Quantifiable

Next steps

- Increase sensitivity
- Expanding NMR database (spectral libraries for more substances)
- Further characterisation of the tobacco smoke condensate (data mining)
- Use NMR as complimentary technique to MS
- Implementation of NMR guided approach for other techniques and method development

Acknowledgements

Giampaolo Venditti, James Donarski

Thank you for your attention…
Questions?