

EXTRA VIRGIN OLIVE OIL VOLATILES A MINE OF CHEMICAL INFORMATION: CHALLENGES IN CHROMATOGRAPHIC DATA ALIGNMENT AND RESPONSE NORMALIZATION FOR RELIABLE FINGERPRINTING BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH TIME-OF-FLIGHT MASS SPECTROMETRY



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Aim and Scope

Comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC×GC-MS) is a highly informative fingerprinting technique for characterization of complex fractions of volatiles (VOCs) in food. In this study, 2D patterns of volatiles from Extra Virgin Olive oils (EVO oils), analyzed over a one-year time-frame by applying chromatographic set-up changes, are evaluated to compensate for 2D peak pattern shifts and response fluctuations to make consistent and reliable the Untargeted/Targeted (UT) fingerprinting process. VOCs from high-quality EVO oils (Italian Violin Project) are sampled by Head Space Solid Phase Micro Extraction (HS-SPME), using a DVB/CAR/PDMS d_f 50/30 μm 1 cm length fiber and analyzed by GC×GC-ToFMS adopting a polar × medium polarity set-up. By altering some chromatographic parameters known to affect 2D pattern characteristics (i.e., modulation period M_p , 2D column dimensions and tuning of TOF-MS parameters), pattern shifts and response fluctuations are registered and evaluated to define a compensation strategy to be adopted in long-time-range studies.

Olive Oil Samples

Violin Project [1]:

- 3 years project
- DOP and IGP Italian EVOOs
- It aims to improve and support oil classification along with valorization of Italian production



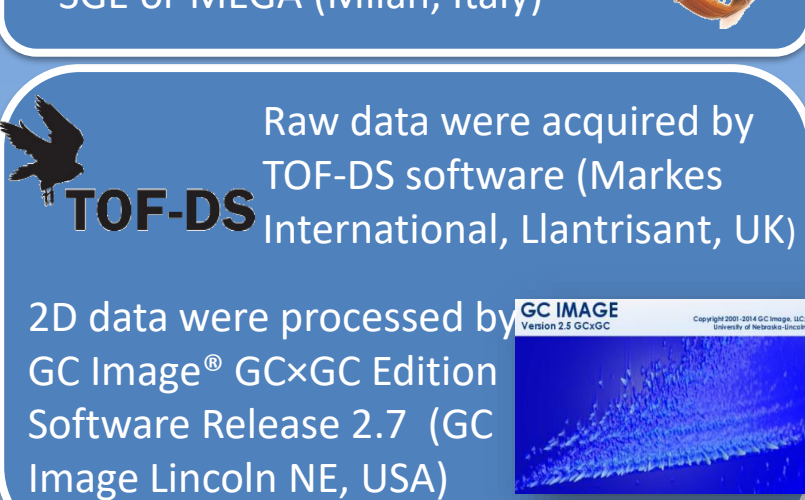
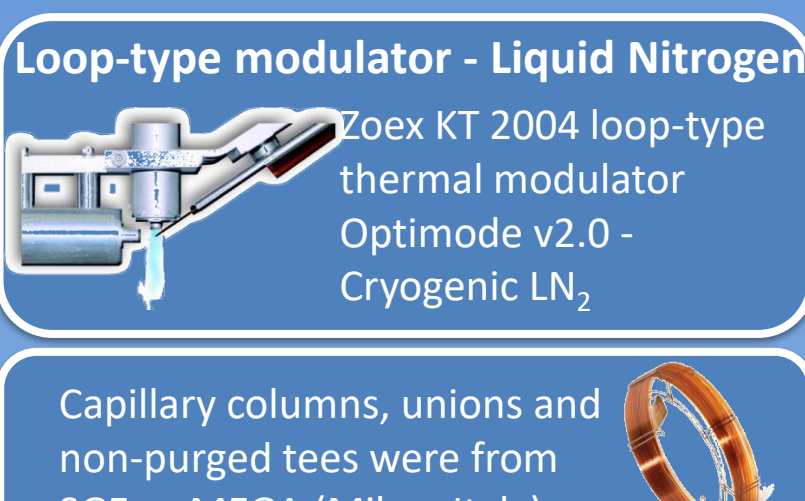
Setup 1

- Modulation period (M_p): 4 s
- 2D column dimensions: 1.80m × 0.01mm ID
- Tuning of TOF-MS parameters for 70eV operations

Setup 2

- Modulation period (M_p): 3.5 s
- 2D column dimensions: 1.45m × 0.01mm ID
- Tuning of TOF-MS parameters for tandem operations (12 and 70 eV)

GC×GC-TOFMS platform



GC Oven programming: 40°C(2') to 240°C(10') @ 3.5'/min
S/SL injector: 250°C Split ratio 1:20
MS Transfer line: 250°C tandem ionization: 70 and 12eV
MS Acquisition: Mass range 40-350 m/z; frequency 50 Hz
Optimode settings: modulation period 3.5-4s, hot-jet pulse 250 ms - cold jet stream MFC from 40% to 5% in 70 min

Headspace Solid Phase Microextraction

Sampling conditions:

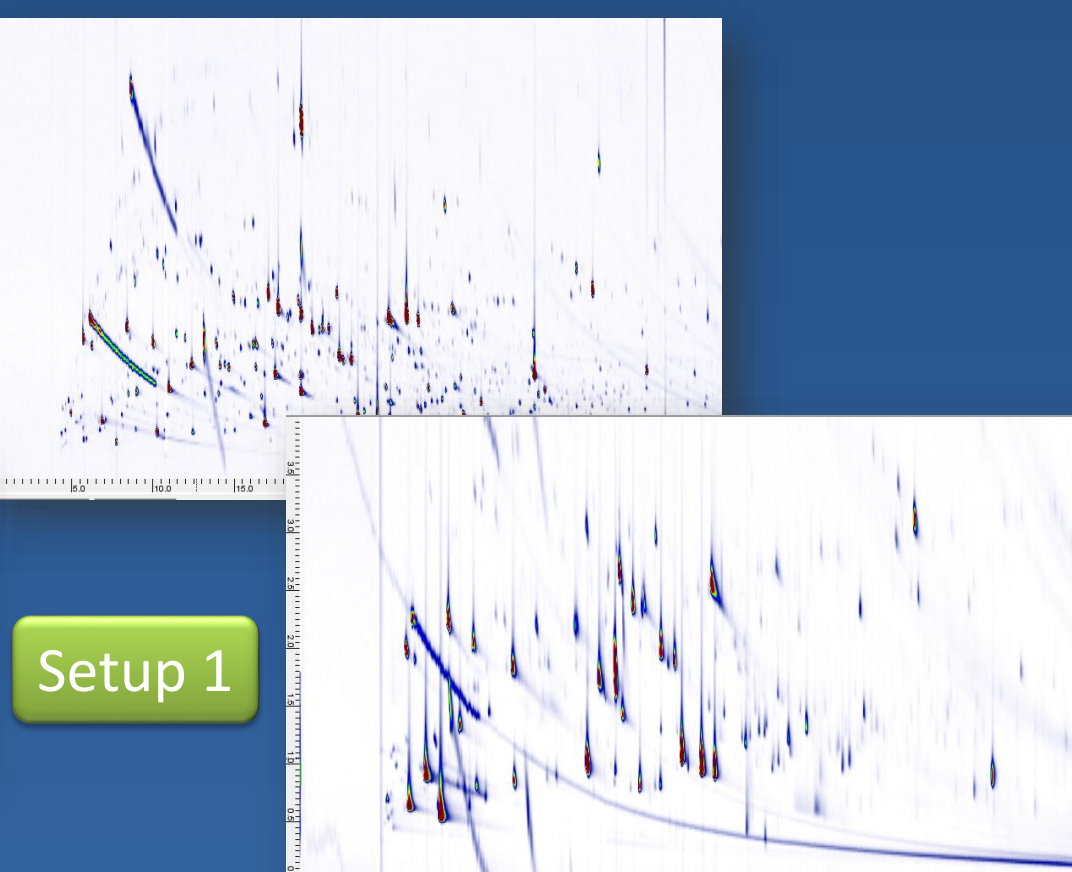
100 mg – 40°C – 60 minutes – 20 ml vial

Checking of the headspace Linearity conducted with Multiple Headspace Extraction (MHE).

The adoption of HS Solid Phase Microextraction with multi-component fiber was optimized for time and temperature to match for headspace linearity conditions and increase fingerprinting sensitivity. Sample amounts below 100 mg avoided saturation effects.

Template transfer and matching

Setup 2
Template 140
target peaks

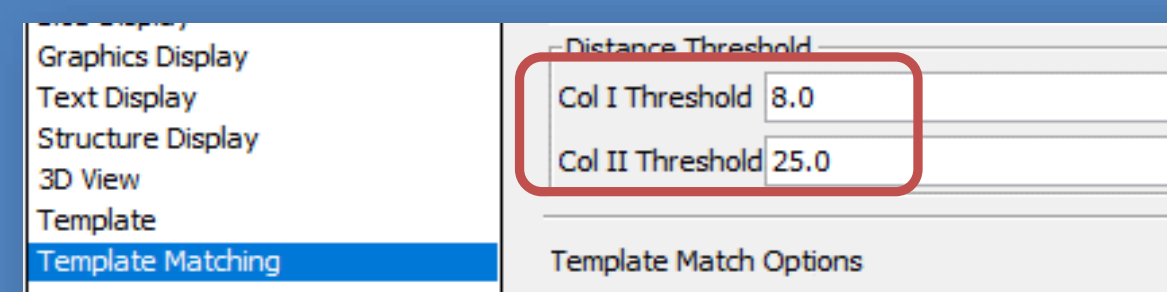


An important step of a comparative process (fingerprinting) workflow that includes analytical runs acquired over a wide time frame (two years or more) is that aimed at comprehensively, yet coherently, mapping detected analytes in a way to enable:

- 1) Full metadata transfer from batch-to-batch (analytes identification, LRI data, Qualifiers and Quantifiers ions, rules for constraints etc...);
- 2) Univocal numbering/ID tagging of single analytes features on adapted templates to enable automatic realignment of data matrices.

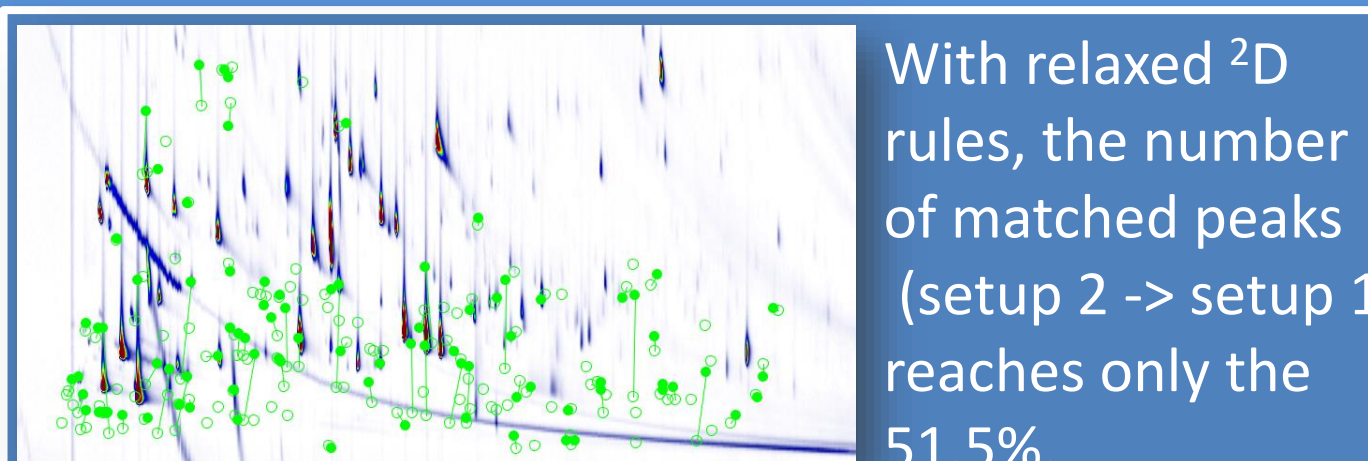
The pattern recognition approaches based on template matching [2] provide smart tools very effective for these operations. However, large time scale variations on chromatographic performances are potential limitation for process effectiveness.

1 Time shift compensation Overtake different M_p



The parameters guiding template matching algorithm are «relaxed» on 2D retention times.

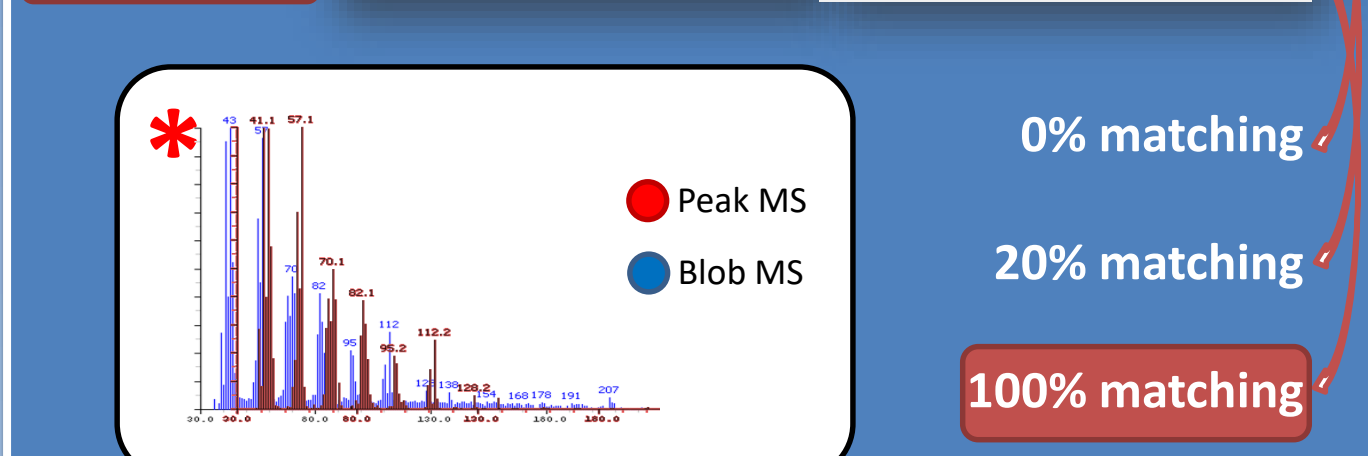
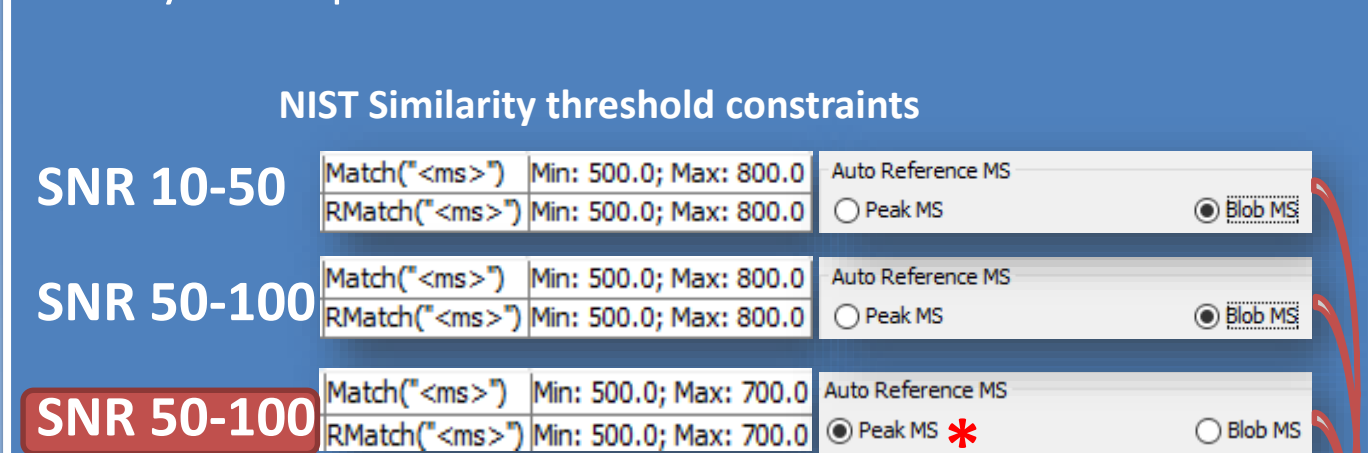
2 Template transform



With relaxed 2D rules, the number of matched peaks (setup 2 -> setup 1) reaches only the 51.5%. A different transform is tested: a "second order polynomial" one is adopted. Any changes to the template matches update the image view and the template transform. After 3-to-5 reiterations the positive matches reach 70%.

3 MSD response fluctuations – confident peaks template

SNR Threshold for reliable template peaks
Comparison of low Signal to Noise Ratio (SNR) peaks between 2 analytical replicates.



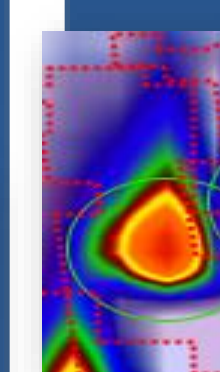
4 Peaks selection

Template peaks SNR >50 → 125 included peaks
90% of information potential kept

Average template matching: **95.4%**

Results and Discussion

UT semi-automatic approach



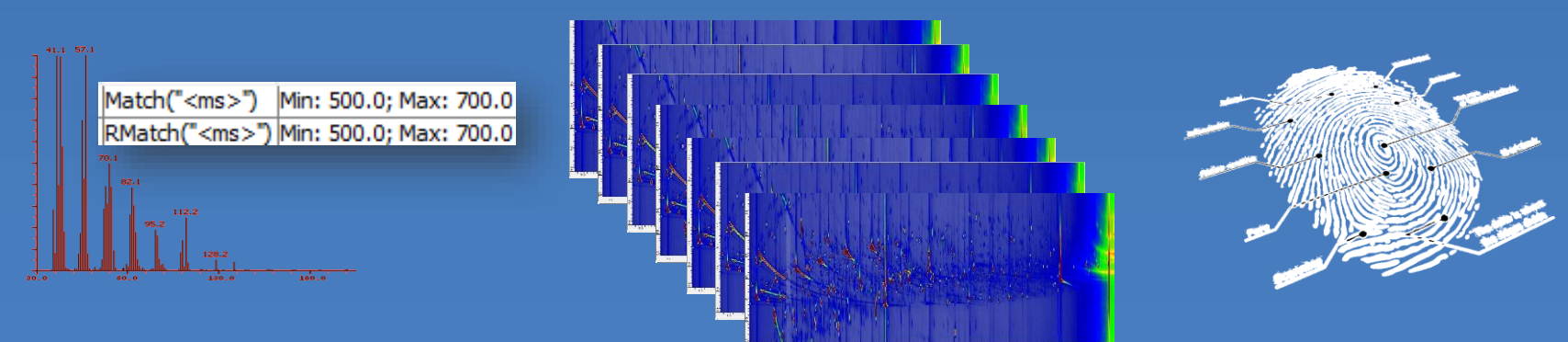
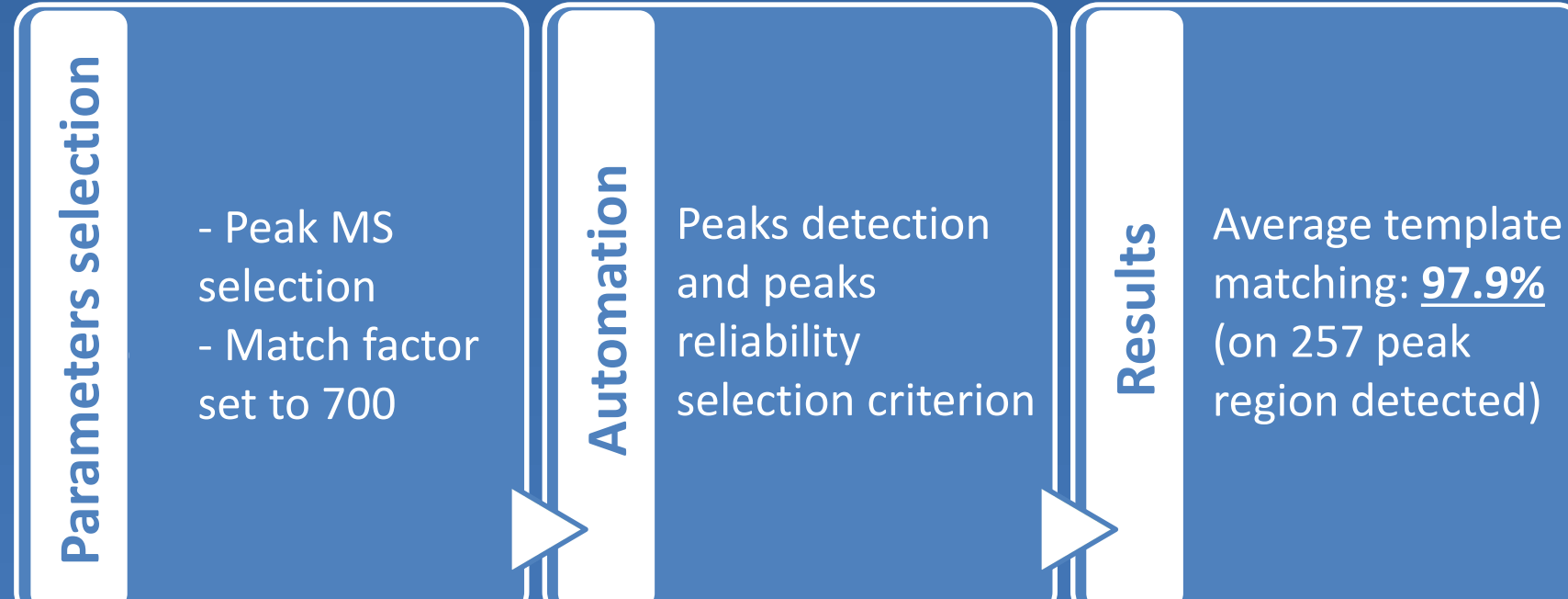
When a template is comprehensively built on 2D peak patterns from a data set by including targeted and untargeted (UT) analytes reliably matched across samples, it enables a truly comprehensive mapping of sample chemical complexity. Template features selection rules can be extended to UT templates for 2D pattern comparison between batches acquired over large time frames.

Targeted template

Building a targeted template come through a fully supervised approach: all reliable 2D peaks are identified through MS spectrum similarity and LRI; 2D peaks metadata are collected to enable template matching limitation rules (Peak or Blob MS choose, MS similarity, Retention Times tolerance, Qualifier and Quantifier ions)

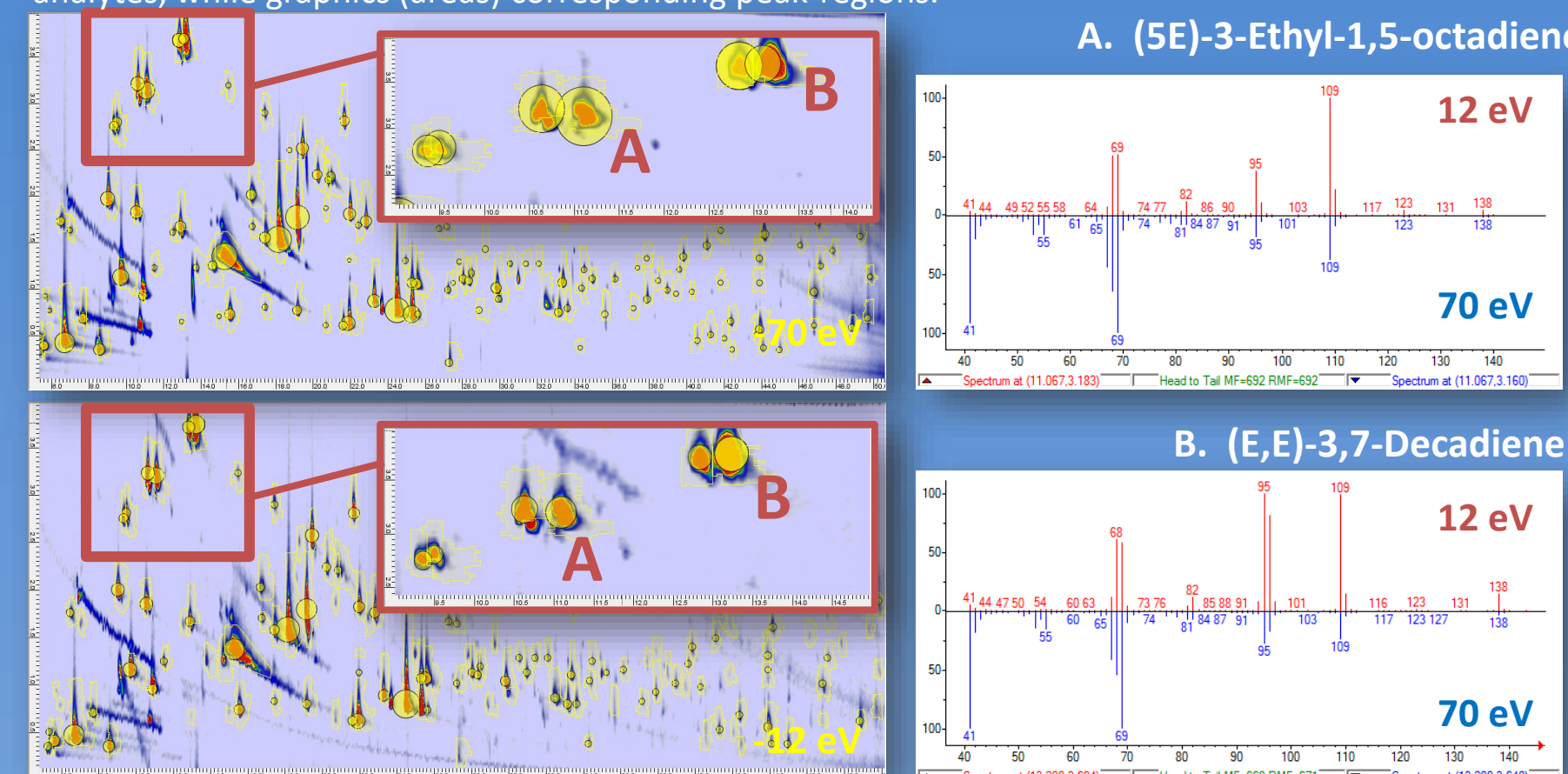
Untargeted template

An untargeted template is capable of comprehensively mapping all 2D peak patterns complexity, also including peaks and peak-regions not a priori identified but capable of carrying out information on sample chemical dimensions.



Exploring the potentials of 12 eV ionization

2D chromatograms of EVOO: yellow circles indicate targeted analytes, while graphics (areas) corresponding peak-regions.



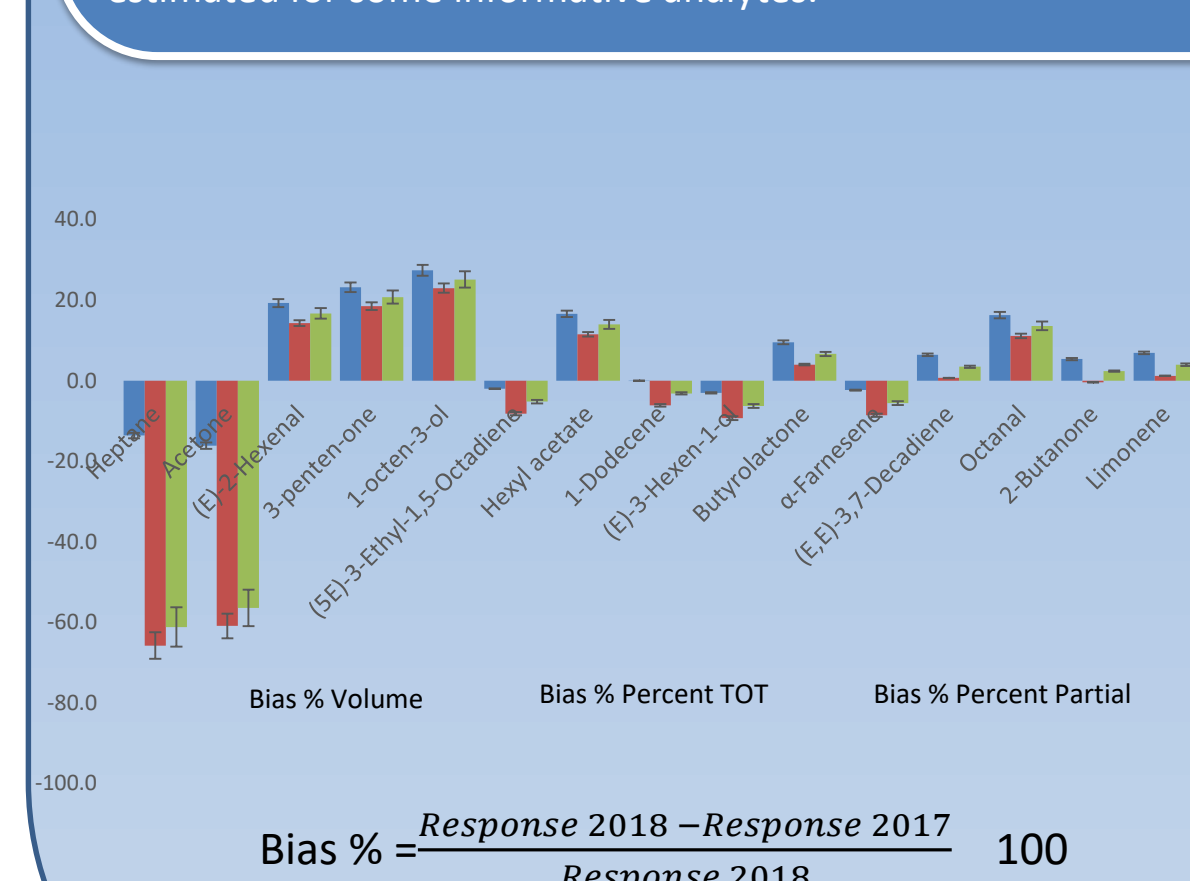
For some compounds, thanks to a generally lower fragmentation, Signal to Noise Ratio (SNR) turn out to be increased in 12 eV compared to 70 eV spectra.

Tandem Ionisation® at 70eV and 12eV offers the possibility to cross-validate in fingerprinting investigations

The availability of an additional MS signature at lower voltages offers the possibility of confirming the identity of analytes not available as reference compounds. Alkenes signature in olive oils is an example.

Response normalization

Response normalization (2D Peaks quantitative descriptors based on TIC or single ions response) is mandatory when data from different analytical session have to be merged and submitted to data mining. In the case of head-space analysis, volatiles are directly extracted by the gas phase in equilibrium (or not) with the condensed sample in a closed system. Response normalization can be done by selecting multiple Internal Standards capable of compensating analytical fluctuations (S/SL injection stability, MSD performances, extra-chromatographic phenomena like analytes adsorption etc.). The following histogram illustrates the % error (bias) between 2D peaks quantitative descriptors (Normalized 2D Volume, 2D Peaks % Response on total image response or on selected UT peaks) estimated for some informative analytes.



Normalization by Internal Standardization performs, on average, less effectively than total image response normalization. However, it better compensates for those response fluctuations derived by S/SL injection discriminations here impacting on highly volatiles due to the different operative head-pressures. On the other hand, % Response data may «mask» variations for less abundant analytes across samples.

Conclusions

This study shows that, thanks to a careful optimization of "smart templates" parameters, it is possible to overcome 2D pattern misalignments and response fluctuations due to chromatographic parameters changes and MS system performances. A full metadata transfer together with univocal numbering/ID of targeted and untargeted analytes are obtained for both fully supervised fingerprinting and automated procedures (i.e. feature templates). Normalization on total image response is a good compromise to response normalization and enables reliable and consistent cross-sample analysis even with pattern misalignments due to chromatographic parameters changes.

References

- [1] G. Purcaro, C. Cordero, E. Liberto, C. Bicchi, L.S. Conte J. Chromatogr. A. 1334 (2014) 101–111.
- [2] F. Magagna, L. Valverde-Som, C. Ruiz-Samblás, L. Cuadros-Rodríguez, S.E. Reichenbach, C. Bicchi, C. Cordero Anal. Chim. Acta. 936 (2016) 245–258.
- [3] Violin Project – Progetto Ager Fondazioni in rete per la ricerca Agroalimentare <http://www.progettoager.it>
- [4] Reichenbach, S. E., Carr, P. W., Stoll, D. R. & Tao, Q. Smart Templates for peak pattern matching with comprehensive two-dimensional liquid chromatography. J. Chromatogr. A. 1216, 3458–3466 (2009)

Acknowledgments

