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# **Diet, lipid peroxidation and colorectal cancer: Targeted UHPLC-ESI-MS/MS analysis of hydroxyalkenal biomarkers in rat serum subjected to different diets**

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Romain Vuillaume, Ndeye Dieng, Loic Mervant, Charline Buisson, Françoise Guéraud, et al.. Diet, lipid peroxidation and colorectal cancer: Targeted UHPLC-ESI-MS/MS analysis of hydroxyalkenal biomarkers in rat serum subjected to different diets. SMAP 2024, Congrès français de Spectrométrie de Masse et d'Analyse Protéomique, Sep 2024, Lille, France. <hal-04719592>

**HAL Id: hal-04719592**

**<https://hal.science/hal-04719592v1>**

Submitted on 3 Oct 2024

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# Diet, lipid peroxidation and colorectal cancer: Targeted UHPLC-ESI-MS/MS analysis of hydroxyalkenal biomarkers in rat serum subjected to different diets.



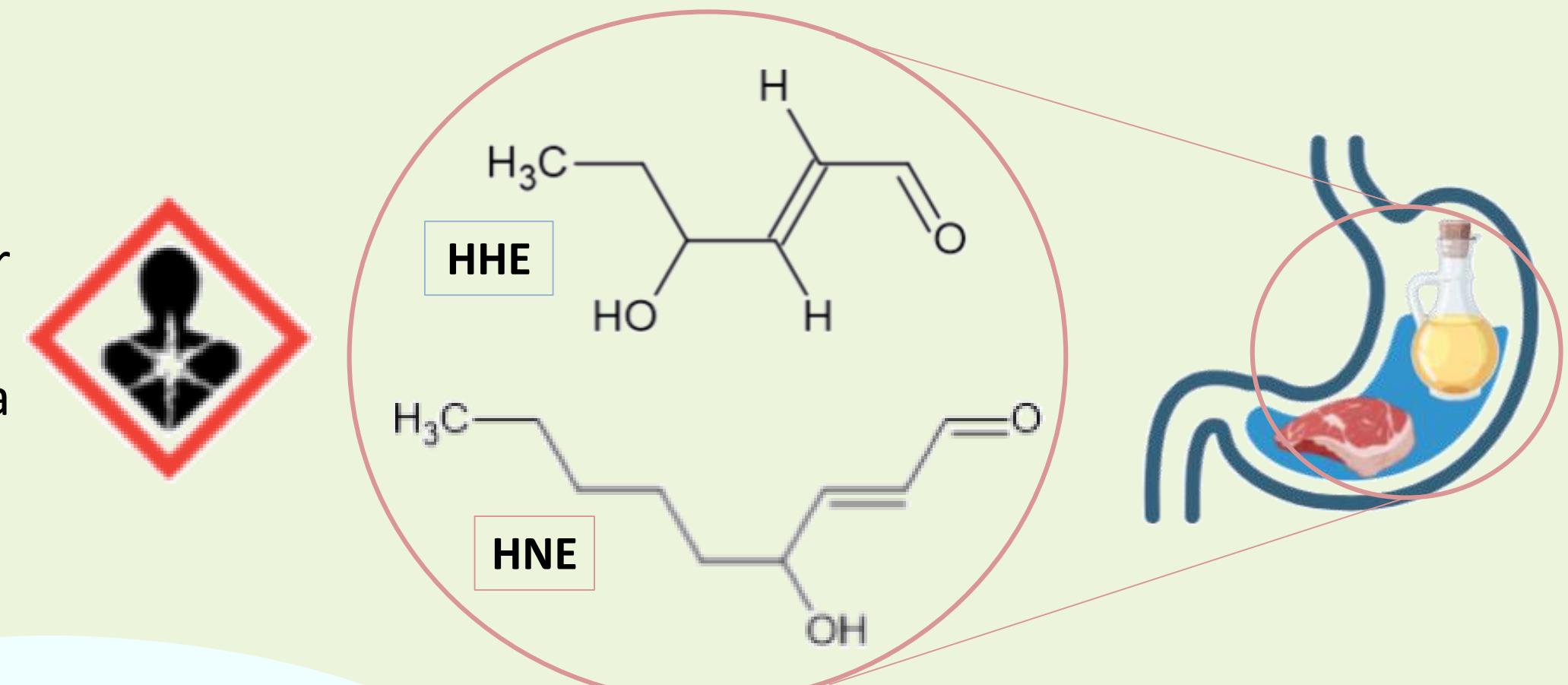
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2 : Metatoul-Axiom Platform, UMR 1331 Toxalim, INRAE, MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics

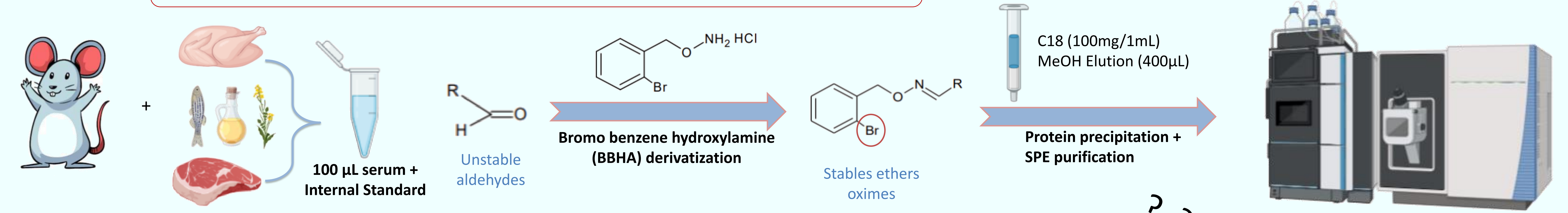
## Introduction:

- Diets rich in red meat are known to be associated with a high risk of colorectal cancer [1].
- Hydroxyl alkenals formed from the peroxidation of polyunsaturated fatty acids catalyzed by dietary heme iron, may be involved.
- These hydroxyl alkenals, in particular 4-hydroxy-2(E)-nonenal (HNE) and 4-hydroxy-2(E)-hexenal (HHE), are known for their reactivity, cytotoxic and genotoxic activities against colon epithelial cells.
- Quantifying these representative hydroxyl alkenals in different diets, during digestion and in biofluids is highly instructive in a toxicological context, enabling correlation with other biological and biochemical markers of colorectal cancer promotion.



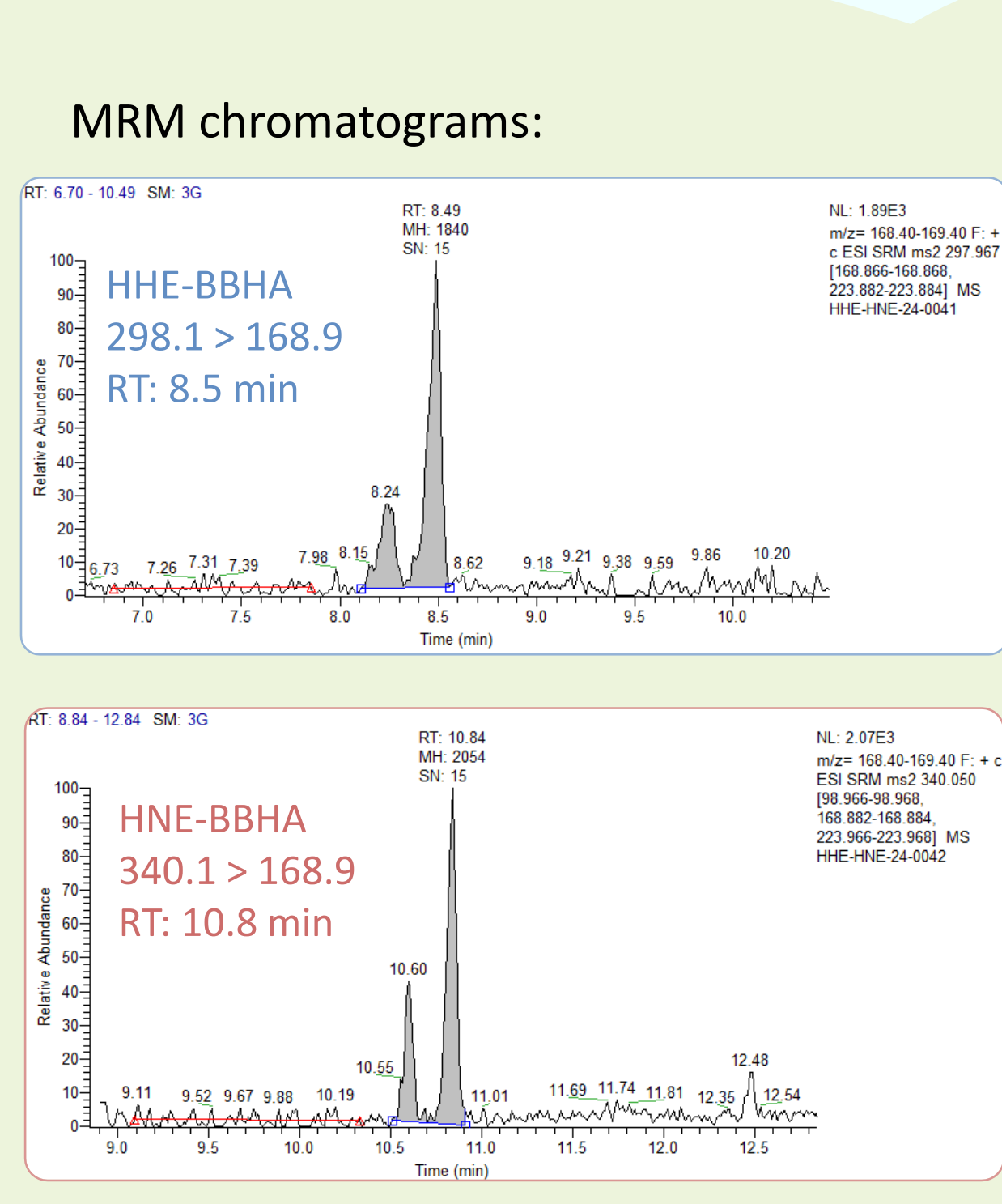
## Methods:

Derivatization with BBHA [2]: > Stabilize the aldehydes  
> Increase the detection selectivity with Br <sup>79</sup> and Br <sup>81</sup> isotopes

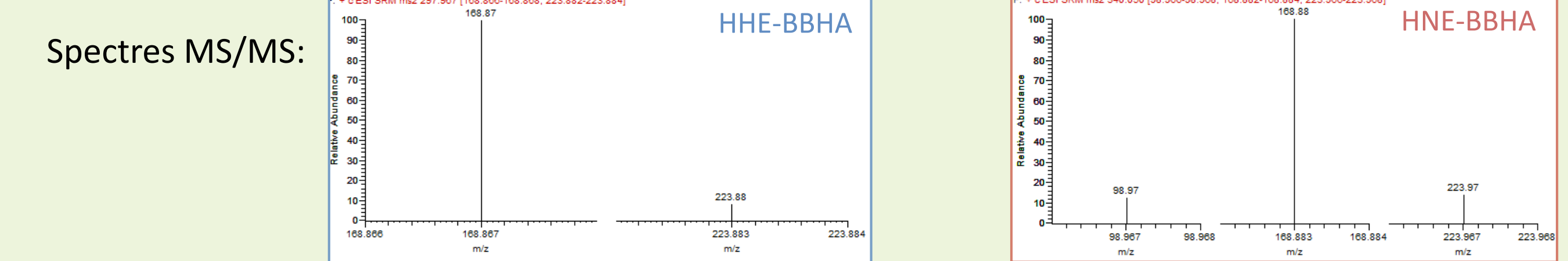


**Experimental design:**  
8 diets:  
- Beef and - Fish oil and - with /without antioxidants (vitamin E and vitamin C)  
- Chicken and - Safflower oil

## MS analysis:



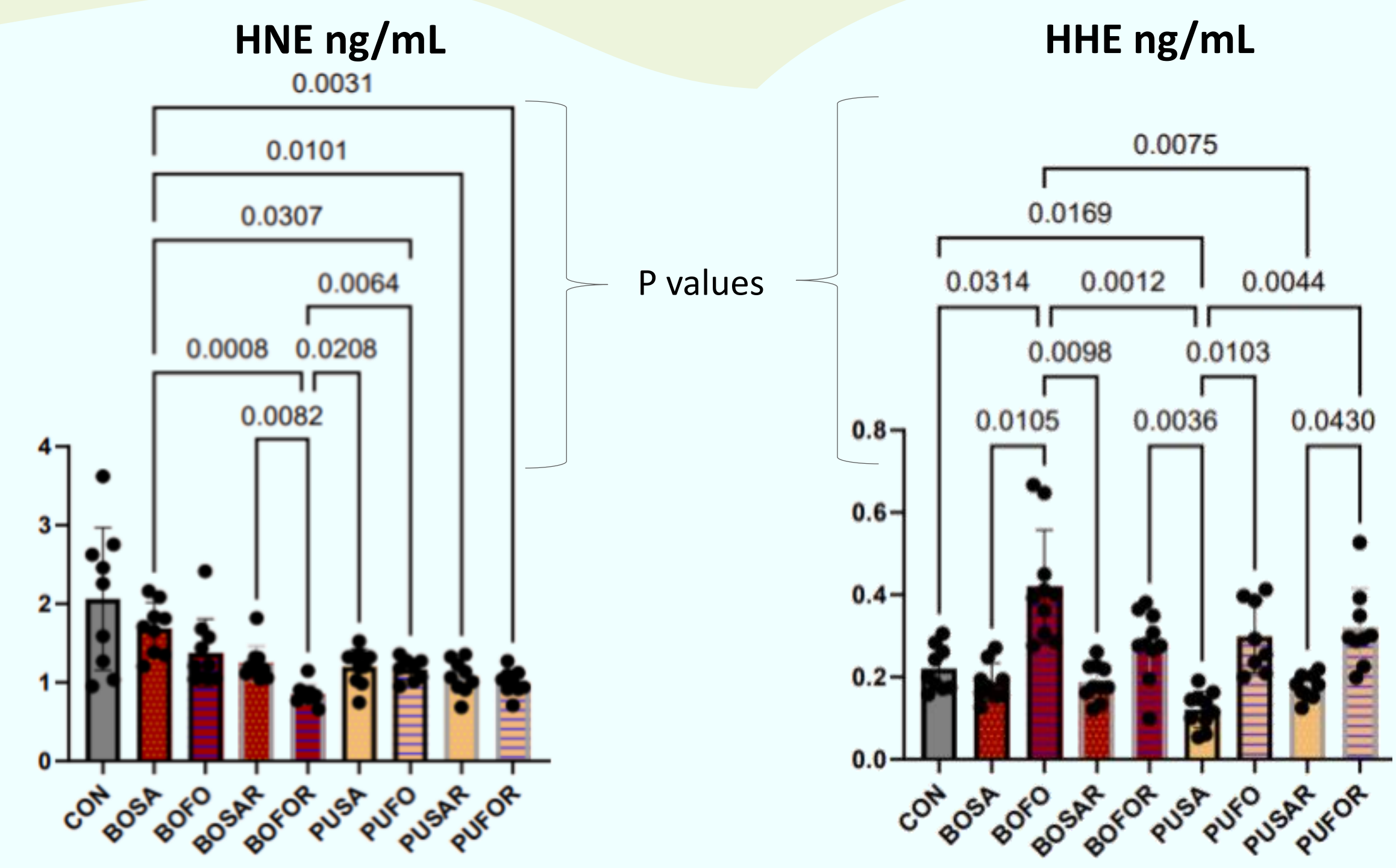
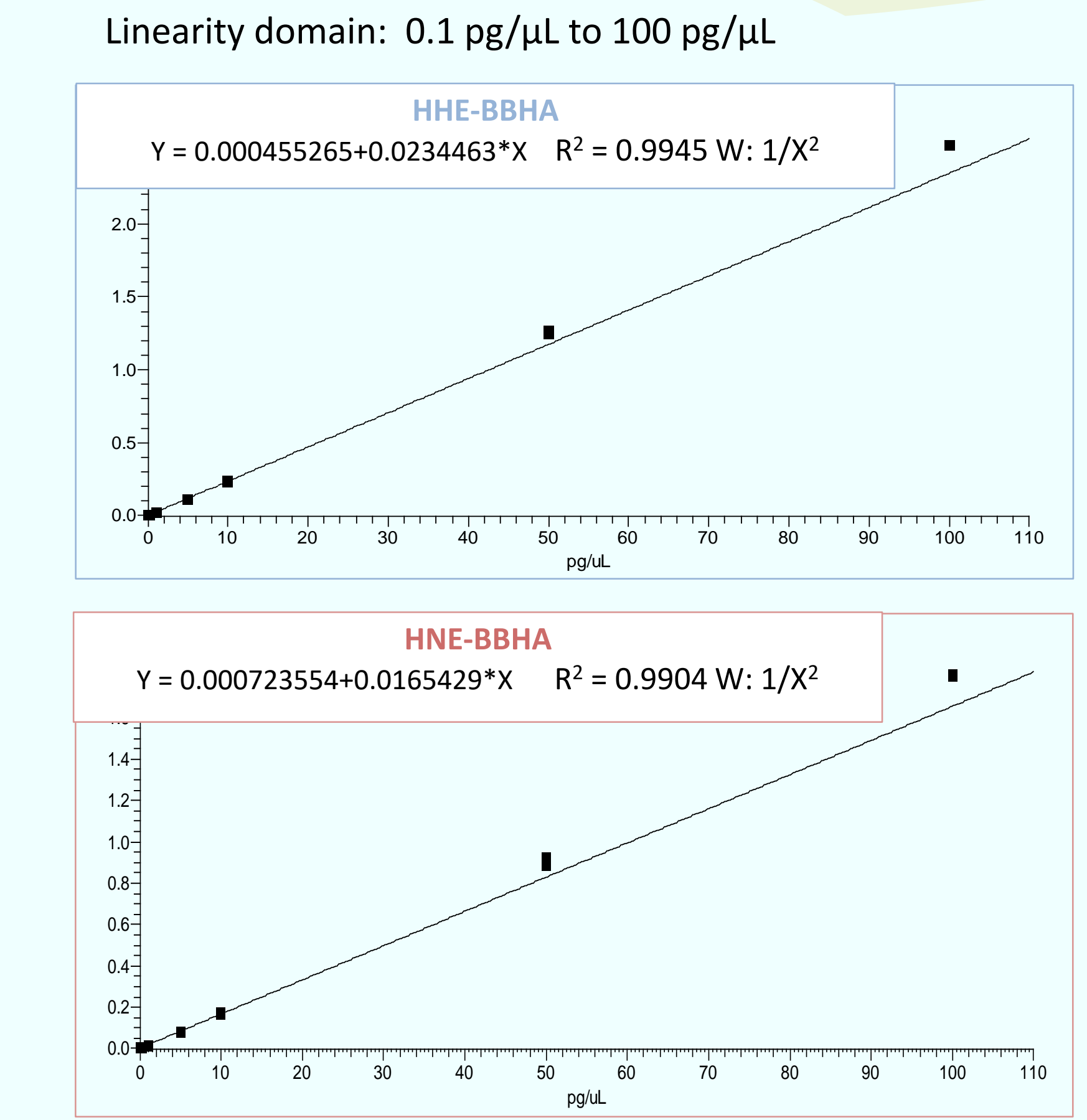
MRM transitions Altis*				MRM transitions Altis*						
Compounds	Parent Ion [M+H] <sup>+</sup> (m/z)	Product Ion	Collision Energy (eV)	Attributions	Compounds	Parent Ion [M+H] <sup>+</sup> (m/z)	Product Ion	Collision Energy (eV)	Attributions	
HHE-BBHA	298.1	169.0	20	C <sub>7</sub> H <sub>6</sub> <sup>79</sup> Br	HNE-BBHA	340.1	169.0	23	C <sub>7</sub> H <sub>6</sub> <sup>79</sup> Br	
		223.9	9	C <sub>10</sub> H <sub>11</sub> N <sup>79</sup> Br			224.0	10	C <sub>10</sub> H <sub>11</sub> N <sup>79</sup> Br	
	300.1	171.0	20	C <sub>7</sub> H <sub>6</sub> <sup>79</sup> Br			342.1	171.0	23	C <sub>7</sub> H <sub>6</sub> <sup>79</sup> Br
		226.0	9	C <sub>10</sub> H <sub>11</sub> N <sup>79</sup> Br			226.0	10	C <sub>10</sub> H <sub>11</sub> N <sup>79</sup> Br	
HHE-d5-BBHA (IS)	303.0	169.0	21	C <sub>7</sub> H <sub>6</sub> <sup>81</sup> Br	HNE-d11-BBHA (IS)	351.0	169.0	24	C <sub>7</sub> H <sub>6</sub> <sup>81</sup> Br	
		225	9	C <sub>10</sub> H <sub>10</sub> DN <sup>81</sup> Br			353.0	225.0	11	C <sub>10</sub> H <sub>10</sub> DN <sup>81</sup> Br
	305.0	171.0	21	C <sub>7</sub> H <sub>6</sub> <sup>81</sup> Br				171.0	24	C <sub>7</sub> H <sub>6</sub> <sup>81</sup> Br
		227.0	9	C <sub>10</sub> H <sub>10</sub> DN <sup>81</sup> Br			227.0	11	C <sub>10</sub> H <sub>10</sub> DN <sup>81</sup> Br	



## LC-MSMS condition on Vanquish Flex + Altis\* (ThermoScientific)

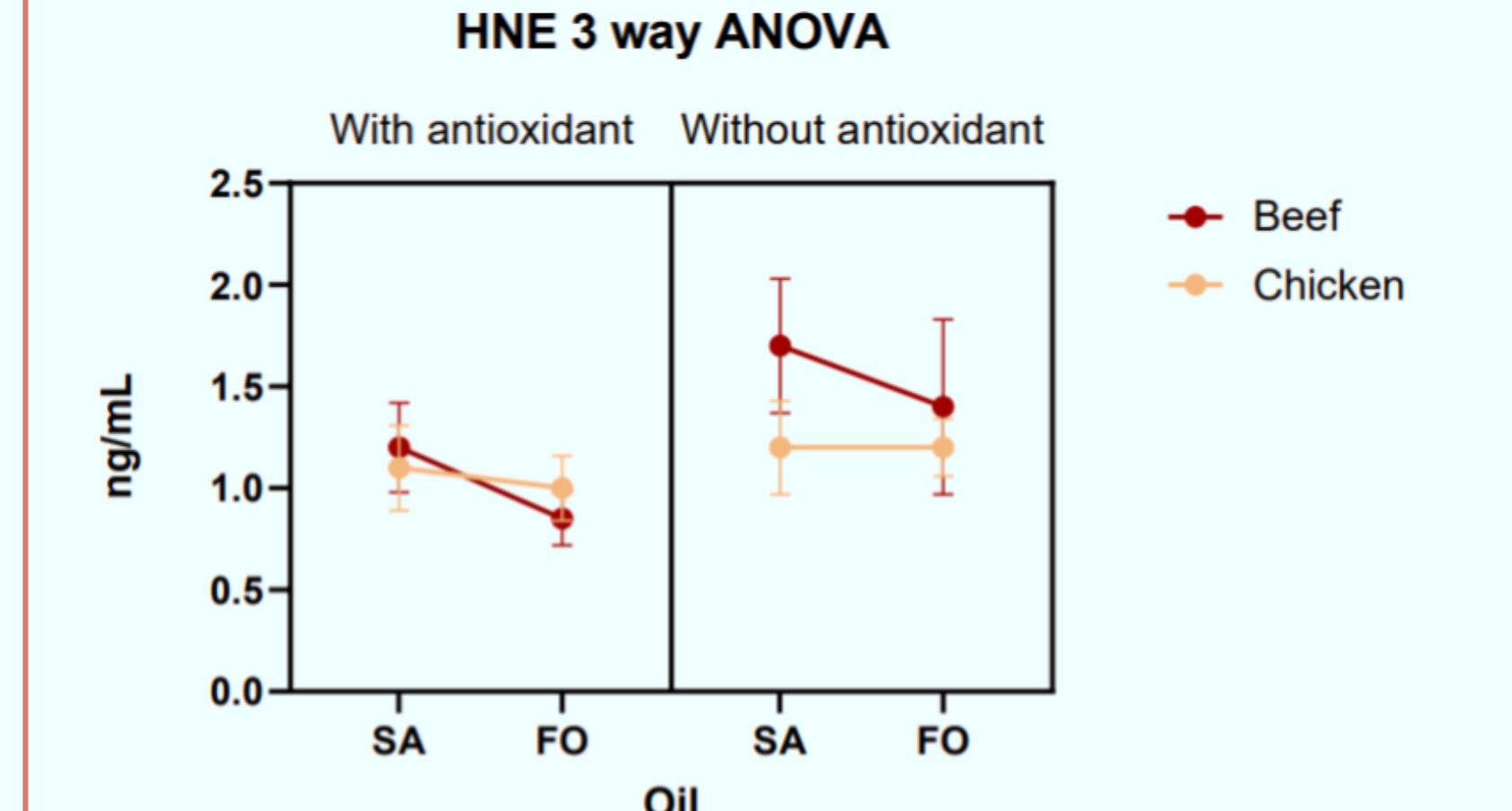
UHPLC column Hypersil Gold C18 (50x2.1mm, 1.9µm)  
Solvent A: H<sub>2</sub>O/ACN 85/15 + 0.1% formic acid  
Solvent B: H<sub>2</sub>O/ACN 5/95 + 0.1% formic acid  
Gradient mode: 0min 0%B, 1min 0%B, 15min 100%B, 17min 100%B, 18min 0%B, 22min 0%B  
Flow: 0.3ml/min. Temperature: 40°C  
Injection volume: 5µL  
ESI +, MRM mode  
Spray voltage: 4000V  
Vaporization temperature: 150°C  
Sheath gas: 20ua, auxiliary gas: 15ua, sweep gas: 0ua  
Capillary temperature: 350°C

## Results:

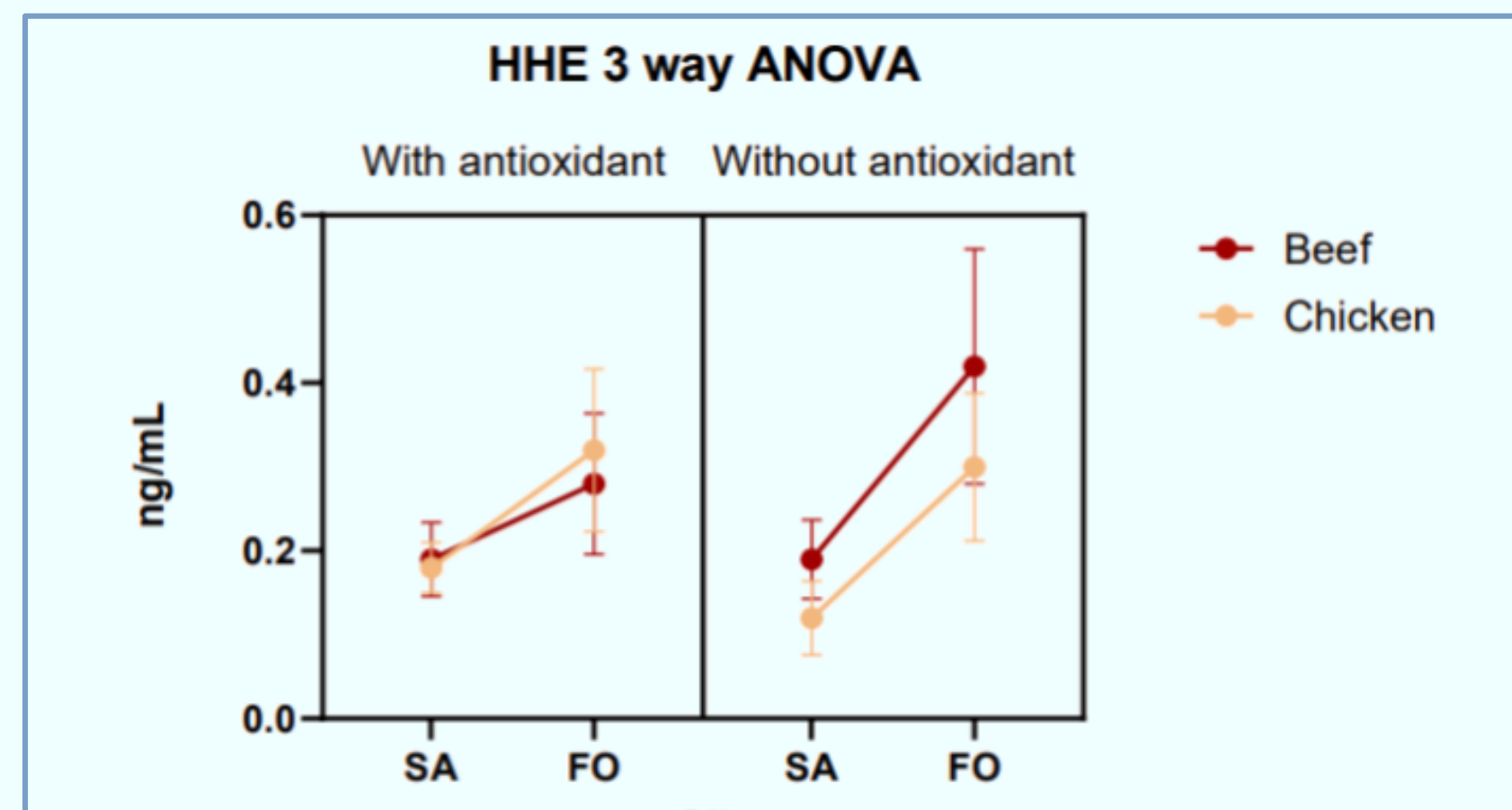


**HNE:** Very significant antioxidant effect  
Very significant oil and meat effect  
Without the antioxidants:  
- Meat effect and no oil effect > Safflower and fish oil contains omega 6 (HNE precursor)  
With antioxidants:  
- Oil effect and no meat effect > Suppressed by the antioxidants

**HHE:** Very significant oil effect > Only fish oil contains omega 3 (HHE precursor)  
Meat effect particularly significant without antioxidants  
Antioxidants suppress meat effect



Source of Variation	% of total variation	P value	P value summary
Oil	7.605	0.0021	**
Antioxidant	24.64	<0.0001	****
Meat	5.712	0.0072	**
Oil x Antioxidant	0.3042	0.5246	ns
Oil x Meat	4.090	0.0220	**
Antioxidant x Meat	7.605	0.0021	**
Oil x Antioxidant x Meat	0.03380	0.8318	ns



Source of Variation	% of total variation	P value	P value summary
Oil	44.49	<0.0001	****
Antioxidant	0.3910	0.4212	ns
Meat	2.780	0.0345	*
Oil x Antioxidant	3.519	0.0179	*
Oil x Meat	7.530e-031	>0.9999	ns
Antioxidant x Meat	5.257	0.0042	**
Oil x Antioxidant x Meat	1.086	0.1820	ns

## Conclusion:

With an original derivatization strategy, stabilized HHE and HNE have been successfully quantified in rat serum. The method has been validated under EMA guideline.  
Significant variations between the diets were observed and can be related to the implication of heme iron in the development of colorectal cancer. It also implies that alkenals originating from the diet and digestion may have an implication outside the digestive tract.



## Acknowledgments:

LCMS analyses were achieved in the French national infrastructure MetaboHUB (MetaboHUB-ANR-11-INBS-0010).

Poster presented at SMAP 2024, Lille, France