

Analysis of oxidized phospholipids by LC (ESI) - MS MS: a lipidomics approach

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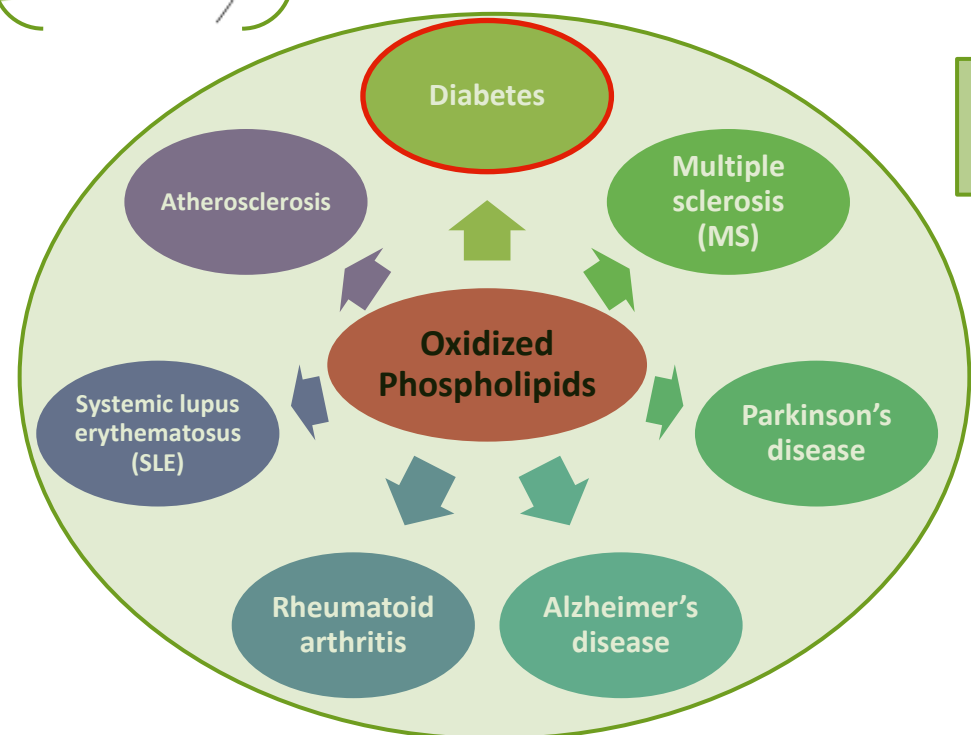
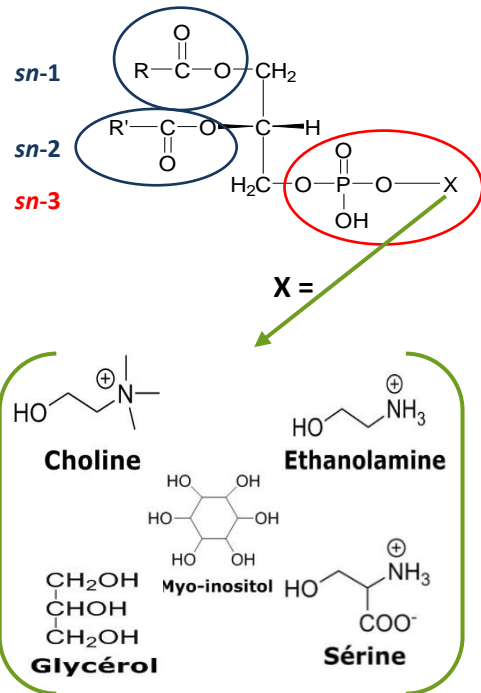
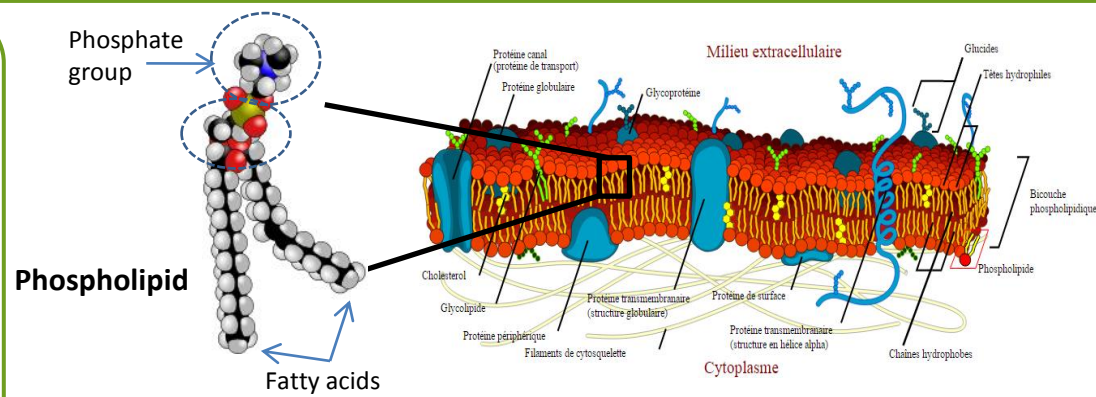
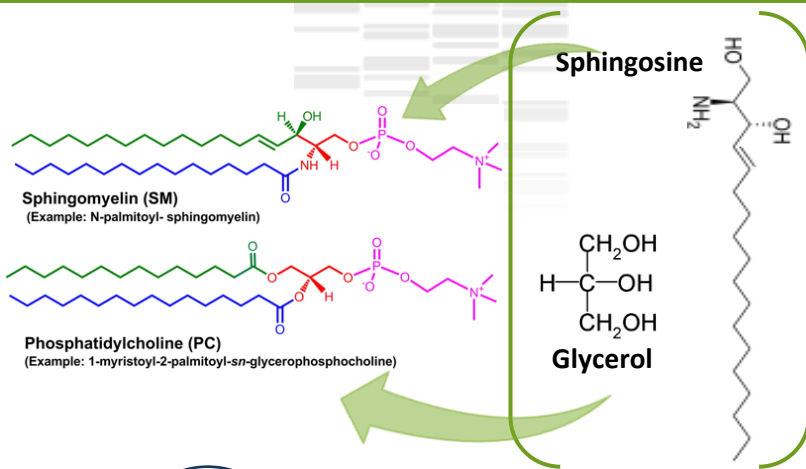
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Flash 10 + Poster 10

Oxidized Phospholipids (PLs-Ox): structure and physiological effects



- Cell membranes
- Lipoproteins

Oxidative stress

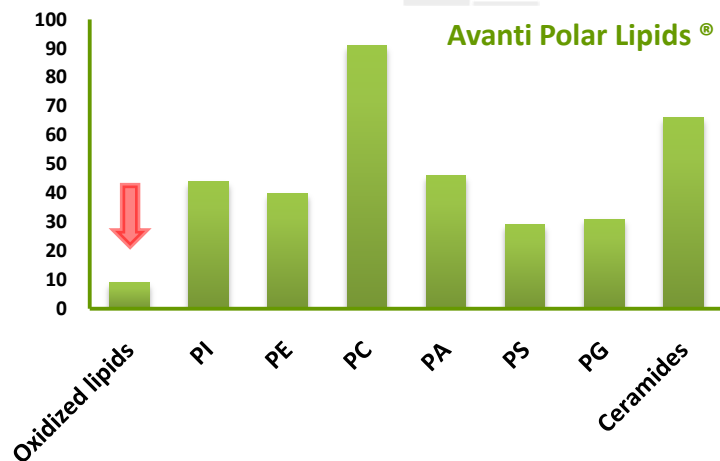
PLs-Ox

PC, PE, SMs,
PS, PI, CL

Fiona H. Greig et al., *Free Radical Biology & Medicine* 52 (2012) 266–280

Analysis of oxidized phospholipids

Commercial availability of Lipids-Ox standards



Synthesis of PLs-Ox

- Chemical Oxidation of PC ($\text{FeCl}_2 - \text{H}_2\text{O}_2$)
- Oxidation of LDL and HDL by CuSO_4
- Oxidation of PC by UVA irradiation
- Oxidation of PC by KMnO_4
- **Autoxidized PAPC**: at room temperature for 5 days to autoxidize
- **Autoxidized PC**: at room temperature (controlled) from **J0** to **J7**

PC(16:0/20:4) - PC(16:0/22:6) - PC(18:0/20:4) - PC(16:0/18:1) - PC(16:0/18:2)

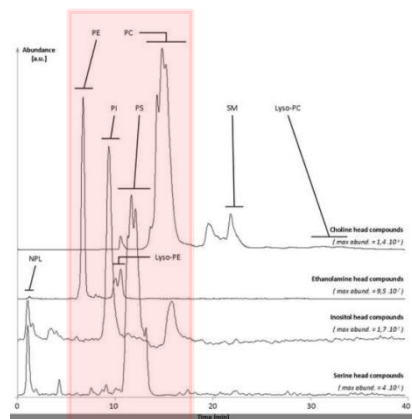
Analytical methods

Hybrid system UHPLC / SFC coupled to mass spectrometry

- Agilent 1260 Infinity SFC/UHPLC System
- AB SCIEX QTRAP® 5500 System

□ Optimization of LC-MS MS method

- Optimization of compound dependent parameters and Source parameters "Electrospray ESI" in (+) and (-).
- Optimization of chromatographic conditions.
- Optimization of MS and MSMS methods.



Phosphatidylcholines (PC)

Negative ionization:

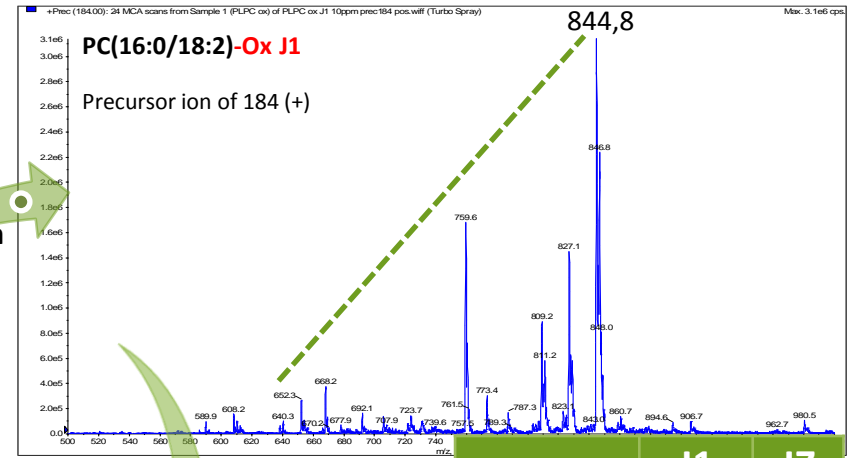
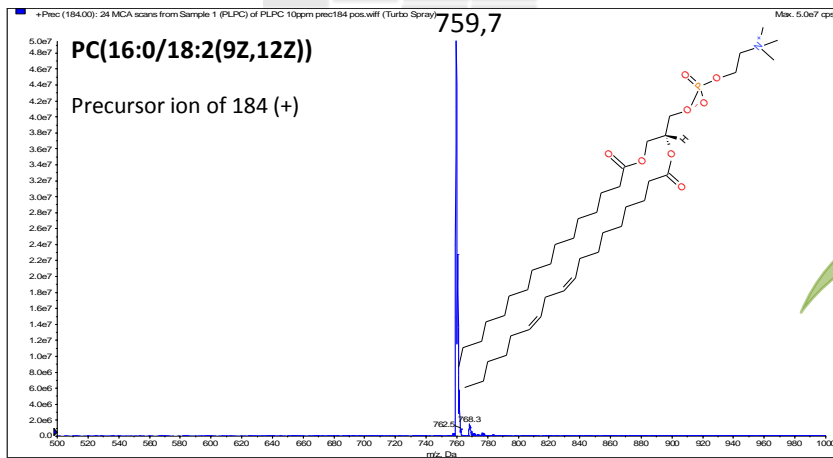
- $[\text{M}+\text{HCOO}]^-$
- $[\text{M}+\text{Cl}]^-$
- $[\text{M}-\text{CH}_3]^-$

Positive ionization:

- $[\text{M}+\text{H}]^+$

- **phase A**: 2-propanol/ H_2O / CHCl_3 /hexane
- **phase B**: 2-propanol/ H_2O / CHCl_3 /hexane
- These phases are supplemented with ammonium formate 12.5 mM

Evaluation of oxidation products of PC(16:0/18:2)



Oxidation

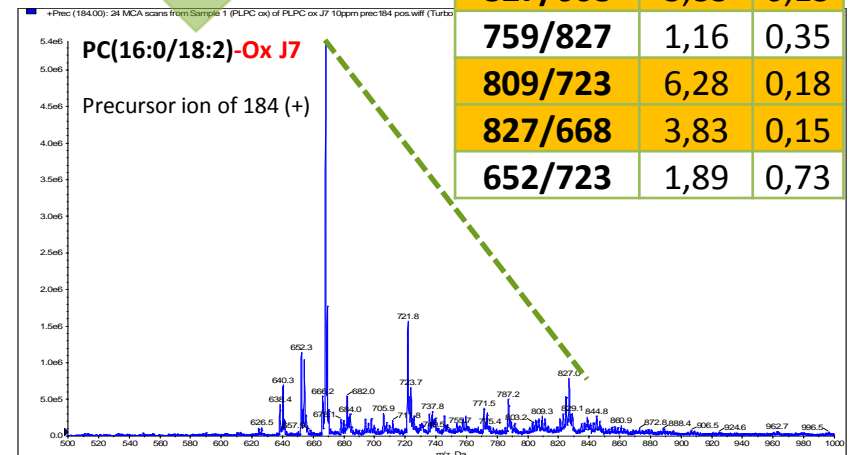
Oxidation

Observed ions	Autoxidation	Chemical oxidation FeCl2
	652,3	
	668,1	
	692	
	707	
	721,8	
	739	
	759	
	774	774
	790	790
	806	806
	827	822
		838
		854

Chemical oxidation
Ana Reis *et al.*, Journal of Chromatography B, 855 (2007) 186–199

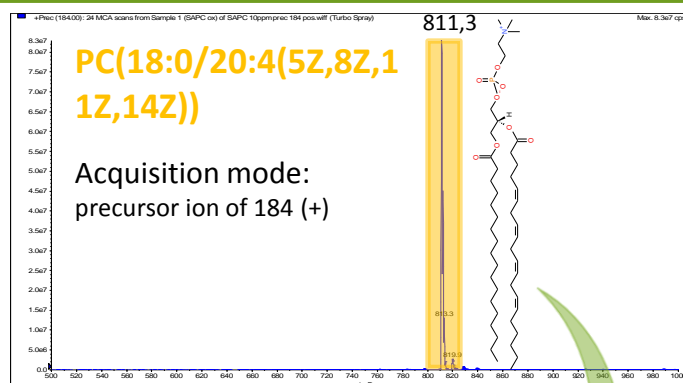
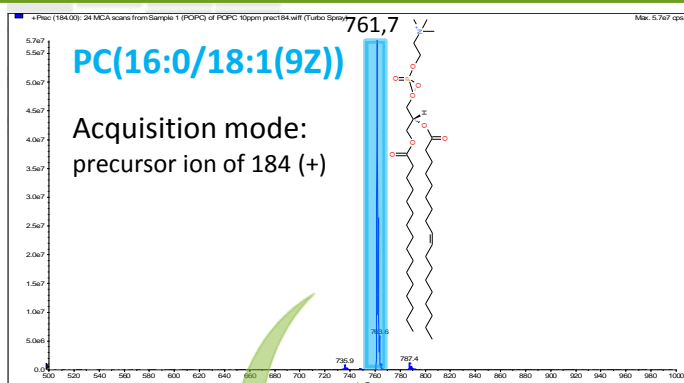
Comparison between observed ions after autoxidation compared to a chemical oxidation

	J1	J7
759/668	4,44	0,05
827/668	3,83	0,15
759/827	1,16	0,35
809/723	6,28	0,18
827/668	3,83	0,15
652/723	1,89	0,73



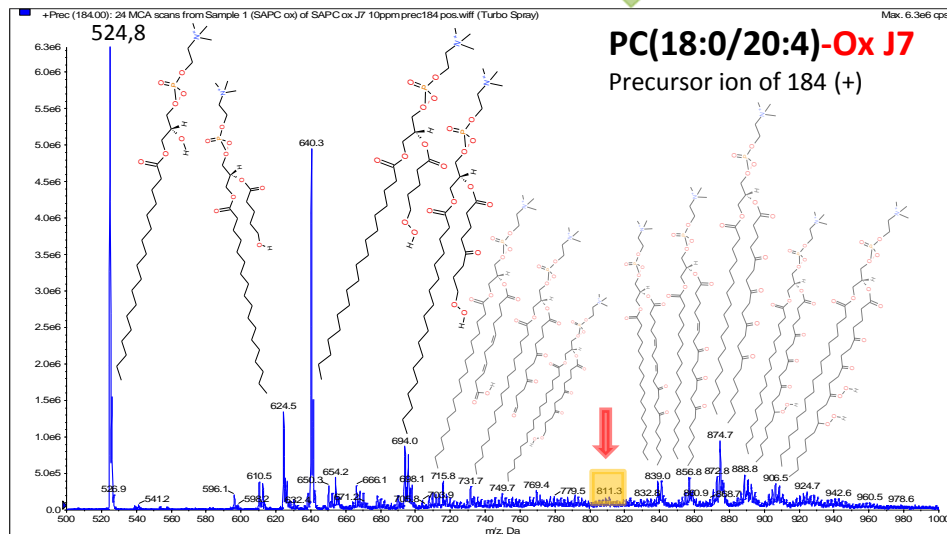
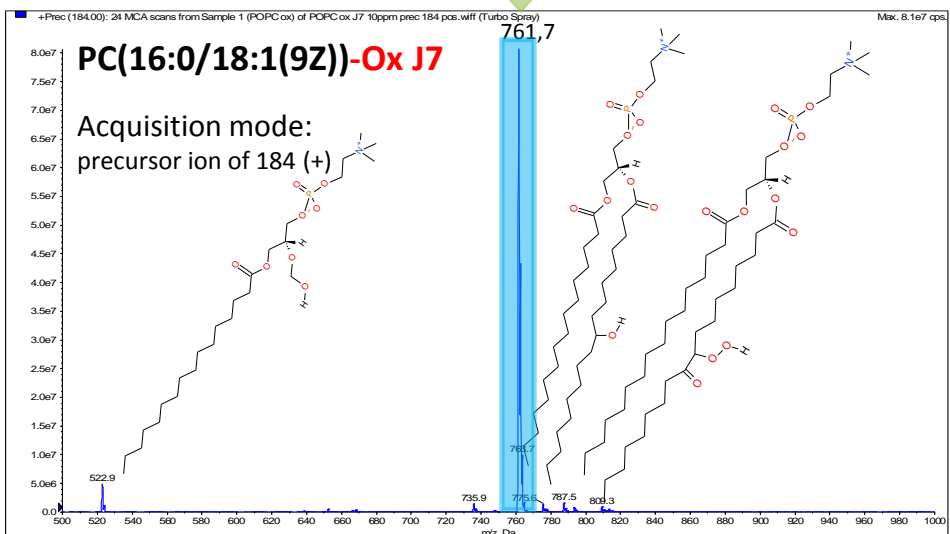
- Variation of intensity ratios of oxidation products according to the oxidation time.
- A larger number of oxidized products is observed in autoxidation vs chemical oxidation.

Oxidation products of two molecular species of PC with 1 and 4 unsaturations



Autoxidation for 7 days

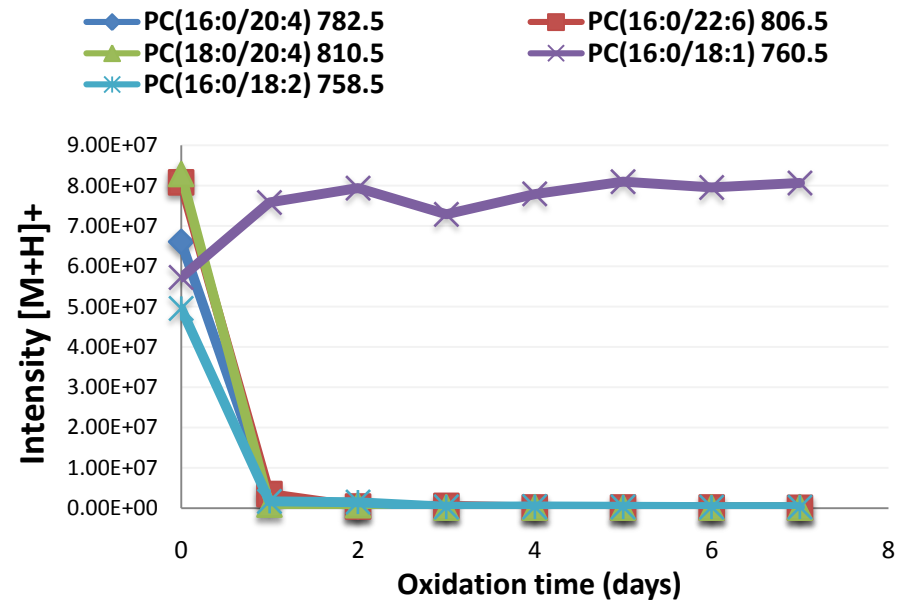
Autoxidation for 7 days



The formation of oxidation products of PLs depends on the structure (double bond)

Conclusion

- Synthesis of PLs-Ox from unoxidized PLs.
- Optimization of the analysis method in LC (ESI)-MS MS using Qtrap 5500.
- Identification of oxidation products of different molecular species of PLs:
 - ✓ Variation of oxidation products depending on the structure of PLs.
 - ✓ Variation of oxidized product ratios according to the duration of oxidation.



Perspectives

- Identification of oxidation products of other phospholipid classes, and also in biological matrices.
- This work will contribute to enrich the database of PLs.