Analysis of triglycerides by UPLC/MSMS. Application in the case of bovine milk fat

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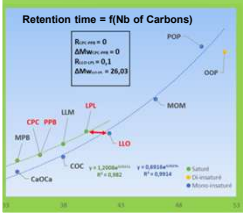
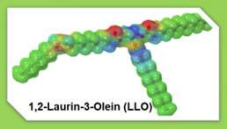
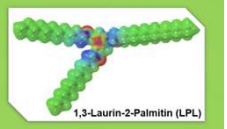
Introduction

The structural isomerism of the various charges around the double bonds of certains TGs moment. This increases thtriglycerides (TG) present in bovine milk makes milk fat one of nature's most complex mixtures in terms of lipid composition.

In addition to structural isomerism, TGs containing unsaturation have a greater velocity than that of saturated TGs, rendering the interpretation of a chromatogram obtained with a conventional detector such as ELSD more difficult. The distribution of electronic charges around the double bonds of certains TGs gives them a higher dipole moment. This increases their relative velocities in the reverse-phase chromatography column.



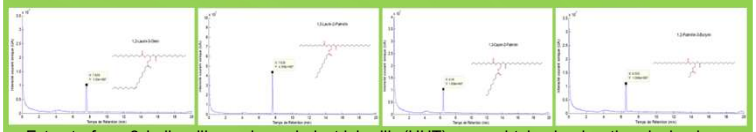




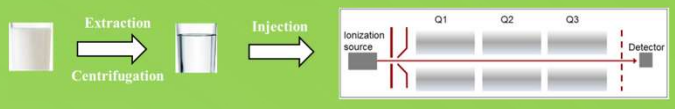
Can the triplequadrupole mass spectrometer with electrospray ionization source analyse the triglyceride composition of milk ? Can it with standards such as LLO, LPL, CPC and PPB identify them in an extract ? That's what we tried to do?

Samples/Materials

Triglyceride standards [1,2-Laurin-3-Olein (LLO), 1,3-Laurin-2-Palmitin (LPL), 1,2-Palmitin-3-Butiryn (PPB), 1,3-Caprin-2-Palmitin (CPC)] of purity greater than 99 %, were used for the experiment.



Extracts from 2 bulk milks and one industrial milk (UHT) were obtained using the single-phase extraction method. The extraction solvent was a 3:5:4 mixture of butanol/methanol/chloroform.



Equipement : UPLC® Acquity coupled with a Quattro Premier XE Mass spectrometer

Experiment

Optimized parameters obtained for the ionization source (ESI+)

	Parent ion (uma)	Capillary (kV)	Cone (V)	Extractor (V)	Adduct (uma)	Formula	MW (uma)	Group
PPB	661,37	4,00	70	3	22,37	$C_{39}H_{74}O_6$	639,00	TG36:0
LPL	712,43	3,60	47	3	17,32	$C_{43}H_{82}O_6$	695,11	TG40:0
CPC	661,37	4,50	61	3	22,37	$C_{39}H_{74}O_6$	639,00	TG36:0
LLO	743,51	3,80	68	3	22,37	$C_{45}H_{84}O_6$	721,14	TG42:0

UPLC® conditions

Acquity UPLC® system with Acquity CSH C18 column 2,1 * 100 mm, 1,7 µm – Temperature : 55°C – Flow : 400 µL/min – Injected volume : 5 µL

Mobile phase A : Acetonitrile/Water (60/40) 10 mM ammonium formate – 0,1 % formic acid

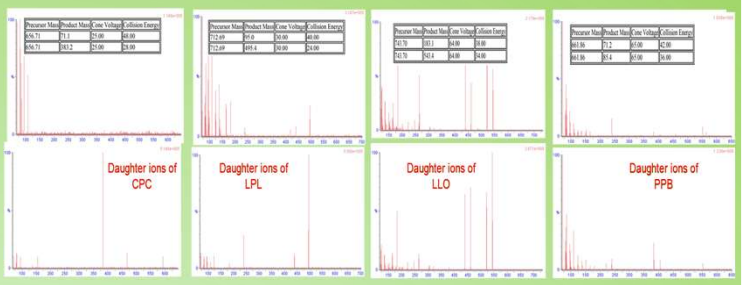
Mobile phase B : Isopropanol/Acetonitrile (90//10) 10 mM ammonium formate – 0,1 % formic acid

Gradient :

Gradient step	Time (min)	% A	% B	Gradient step	Time (min)	% A	% B
1	-	60,0	40,0	4	18,00	99,0	1,0
2	3,00	46,0	54,0	5	18,10	60,0	40,0
3	3,10	70,0	30,0	6	20,00	60,0	40,0

Mass spectrometry conditions (obtained by the autotune system, syringe flow rate : 50 µL/min)

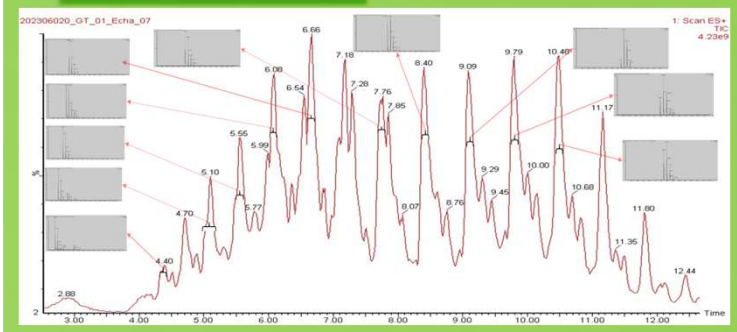
MRM parameters for the four standards used to characterize the milk



MS Scan parameters :

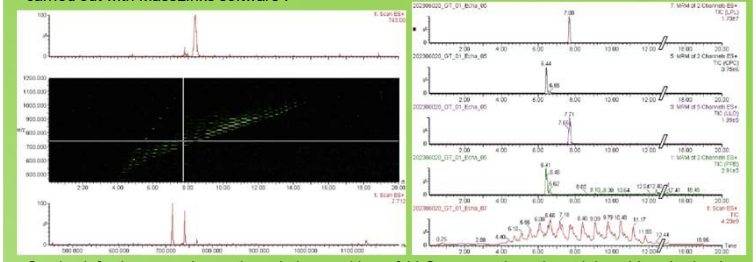
ESI+ - Mass range from 250 to 1200 uma (m/z) – Capillary : 4,30 kV – Cone : 52 V – Extractor : 4 V – Source temperature : 120 °C – Desolvation temperature : 350 °C – Cone gas : 120 L/hr – Desolvation gas : 900 L/hr

Results



Chromatographic profile of large-mix milk and corresponding mass spectra at intervals of a few seconds. The chromatograms of raw large-mix milk and industrial milk show similarities. The majority of bovine milk seems to be represented by a few triglyceride groups (highest peak).

The complexity of the triglycerides composition is even more highlighted thanks to the mapping carried out with MassLinks software :



On the left, the map shows the relative position of LLO among the other triglycerides. It clearly shows the co-elution of TGs (green traces on the map) of different masses.

On the right, we can see that the MRM method for the detection of LPL, LLO and CPC is justified by the number of TGs present in milk. In the case of PPB, the daugther fragments have masses on charges of less than 100 daltons. Fragments of other TGs may correspond to these daughter fragments, compromising their identification. In the PPB's mass spectrum, the mass of PPB appears regularly at different times, unlike its isomer CPC.

Conclusions

The phenomenon observed with a detector such as ELSD for LLO and LPL is wel represented here, with larger masses being able to elute with smaller masses. Triple quadrupole mass spectrometer allws visualization of the complexity of the triglyceride composition of milk. This orthogonal dimension of the chromatograms is essential for the discrimination of TGs. The use of guaranteed purity standards is also necessary for the identification of triglycerides.

The MRM mode wich allows a double selectivity, on the parent ions and the daughter ions, offers the possibility of discrimating between isomers. In this Mode both analysersca be set to constant voltages and improve their sensitivities. This mode of operation can allow the quantification of target molecules.

Studies have shown that certain specifics groups of TGs can have a significant impact on the characteristics of dairy products such as texture, taste, digestibility. But, characterizing the triglyceride profile of milk is a longand expensive process. The implementation of a tool capable of quickly identifying and quantifying these groups would give a definite advantage in the orientation and valuation of dairy products.

If the quantification of triglycerides is possible in milk with UHPLC-MSMS, then it would perhaps be possible, by combining mathematical tools wiht the chemical data obtained, to create prediction models based on the infrared absorption spectra of milk from cow, area in wich the Knowledge and Valorization of Agricultural Products Department of the CRAW has an international expertise.

Perspectives

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