Laboratorio Accreditato EN ISO/IEC 17025 da Accredia
Numero di accreditamento: 1786L

09/01/2023 Annex Test Report FC220857.01

1. HPLC/QTOF screening of non-volatile substances

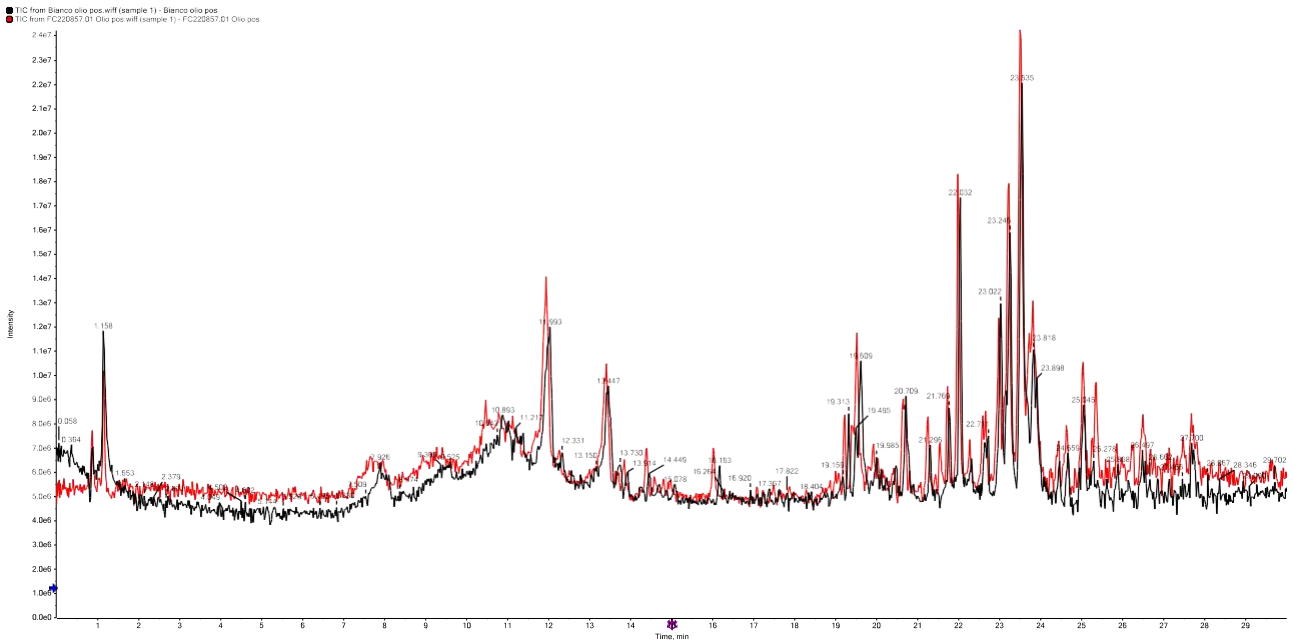
LC-HRMS screening is performed by the laboratory with Shimadzu Nexera X2 UHPLC instrumentation coupled with a high-resolution QTOF Sciex 4600 mass spectrometer.

Using the LC-Q-TOF analytical technique, it is possible to detect pollutants present in matrices of various kinds, at very low concentrations.

This technique performs a first chromatographic separation of the analytes through the UHPLC system. Subsequently, the analytes are desorbed by the ESI (electron spray ionization) source and enter the QTOF system, where they are further separated from the first quadrupole based on their mass/charge ratio and finally from the time-of-flight analyzer, which allows to obtain a high resolution of molecular ions allowing the identification of the brute formula. All ions are acquired in both positive and negative mode with a charged mass ratio between 50 and 1200 Da.

Positive mode

FIG. 1 Overlay TIC Blank (black line), FC220857.01 (red line)

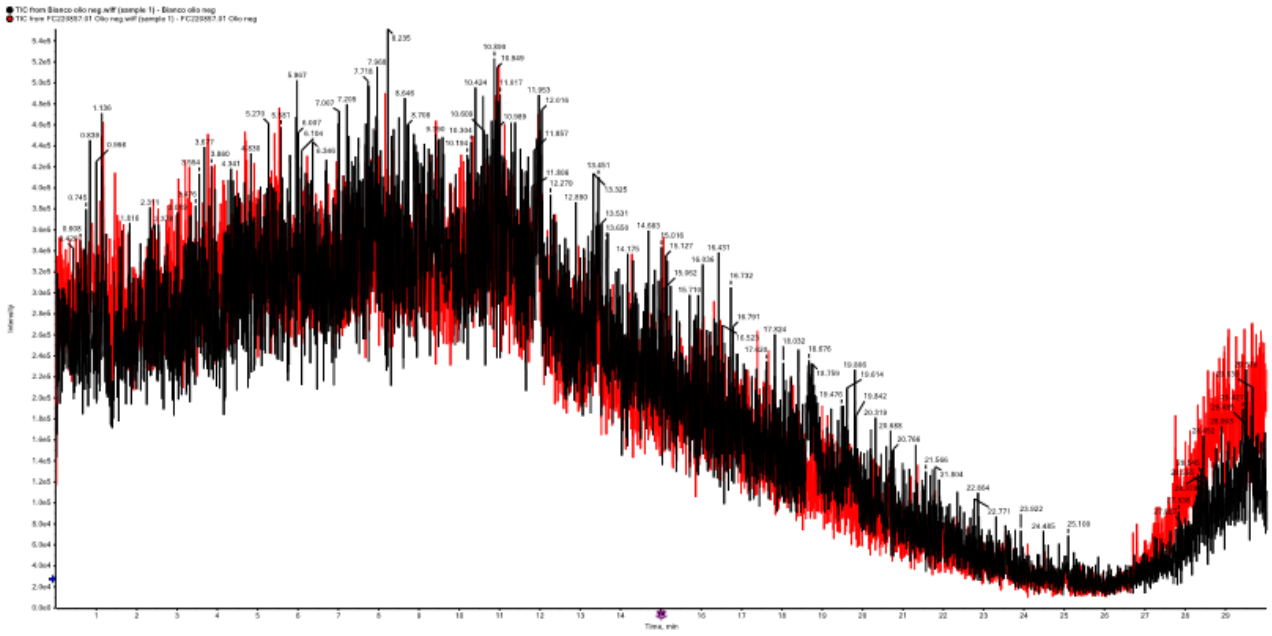


In positive mode, no differences were found between the process blank and the food simulant after contact with the sample for 6 minutes at 500 W in the microwave.

Laboratorio Accreditato EN ISO/IEC 17025 da Accredia
Numero di accreditamento: 1786L

Negative mode

FIG. 1 Overlay TIC Blank (black line), FC220857.01 (red line)

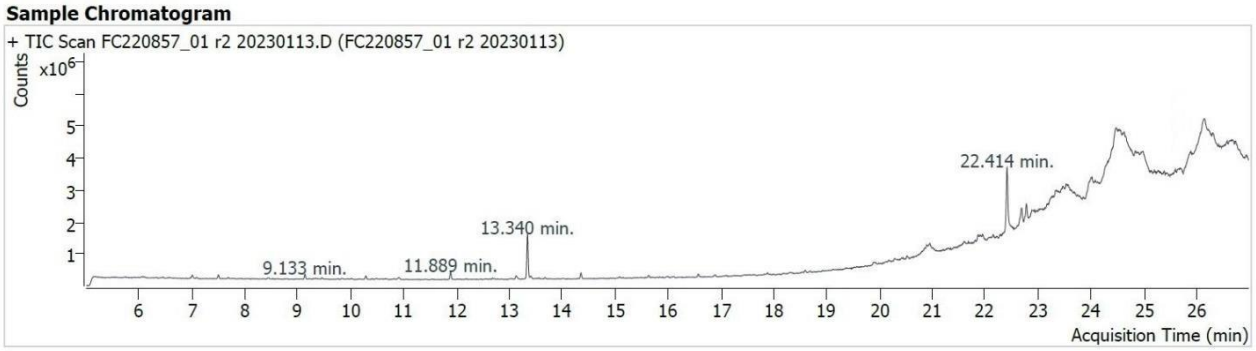


In the negative mode as well, no differences were found between the process blank and the food simulant

1. GC-MS Screening

The Laboratory carries out the analysis in GC-MS, in order to identify volatile and semi-volatile, non-polar compounds, in line with the analytical and scientific principles of risk assessment internationally recognized. The screening tests performed with GC-MS technique compare the quantities of analytes with internal standard, making a semi-quantitative evaluation. The quality recognition is operated through the NIST library supplied with the instrument; The comparison between the mass spectra detected and those in the library is expressed as a percentage probability of matching. The laboratory proposes as LOI (level of interest) a quantity of analyte in simulant / food equal to 90ug / kg (Comsas strategy) and as recognition a match greater than 80% provided by the comparison with NIST. Screenings were performed with GC MS Shimadzu and QP2010SE GC MS TQ Agilent 7000E instrumentation.

1.1 Screening in GC/MS FC220857.01



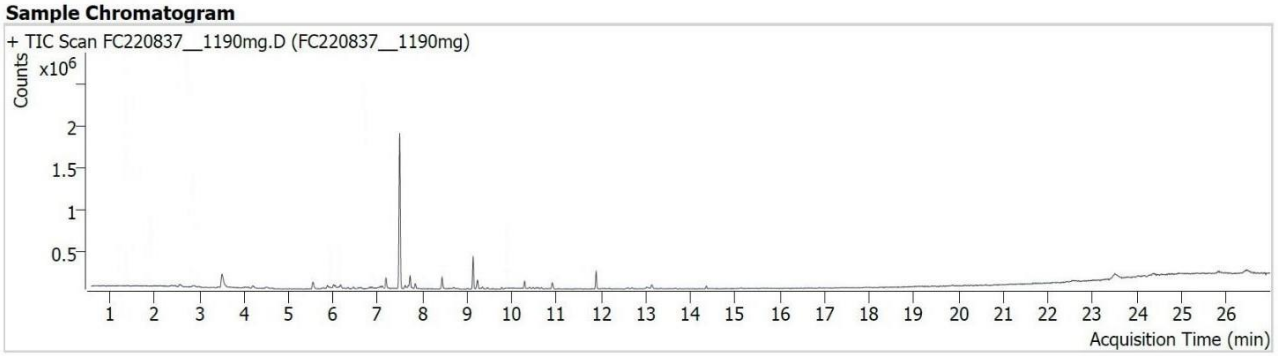
Retention time (min)	**Peak identification**	**Instrumental semi-quantitative evaluation (mg/kg)**	**CAS**	**Cramer class**	**Reg. 10/2011 (mg/kg)**
7.499	3-Phenylpentane (**standard interno**)	0.10	1196-58-3		
9.133	Nonane, 3,7-dimethyl-	0.10	17302-32-8	I	-
11.889	Decane, 2,4-dimethyl-	0.15	2801-84-5	I	-
13.340	2,4-Di-tert-butylphenol	1.18	96-76-4	I	
22.414	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	1.13	119-47-1	-	60

Note: Cramer I class Lim. 1.80ppm; Cramer II class Lim. 0.54ppm; Cramer III class Lim. 0.09ppm.

Laboratorio Accreditato EN ISO/IEC 17025 da Accredia
Numero di accreditamento: 1786L

1.2 Screening HS GC/MS FC220857.01

Sample Chromatogram



Retention time (min)	Peak identification	Semi-quantitative evaluation Content mg/kg	CAS	Cramer class	Reg. 10/2011 (mg/kg)
7.49	3-Phenylpentane (standard interno)	0.1	1196-58-3	_	_
7,18	Acetophenone	0.01	98-86-2	I	_

Note: Cramer I class Lim. 1.80ppm; Cramer II class Lim. 0.54ppm; Cramer III class Lim. 0.09ppm.