

Electronic Supplementary Information

Supplemental Table 1. Fecal and urine content of ellagic acid and urolithin A, B analyzed by HPLC.

	Baseline				Week 4			
	Fecal	Fecal	Fecal	Urine	Fecal	Fecal	Fecal	Urine
	EA	UA	UB	UA	EA	UA	UB	UA
	µg/g stool	µg/g stool	µg/g stool	µg/mg creatinine	µg/g stool	µg/g stool	µg/g stool	µg/mg creatinine
Non- Producers	0-6.4	0	0	0-2.9	0-174.2	0	0	0-0.6
Producers	0-3.7	0-71	0-36	0.1-28	0-19	14-317	0-85	2.8-36

Supplemental Table 2. Fecal content of punicalagin A/B and punicalin analyzed by HPLC.

		Baseline		Week 4
	Fecal	Fecal	Fecal	Fecal
	Pun A/B	Punicalin	Pun A/B	Punicalin
	µg/g stool	µg/g stool	µg/g stool	µg/g stool
Non- Producers	0	0	0-49	0-7.4
Producers	0	0	0-27	0-5.7

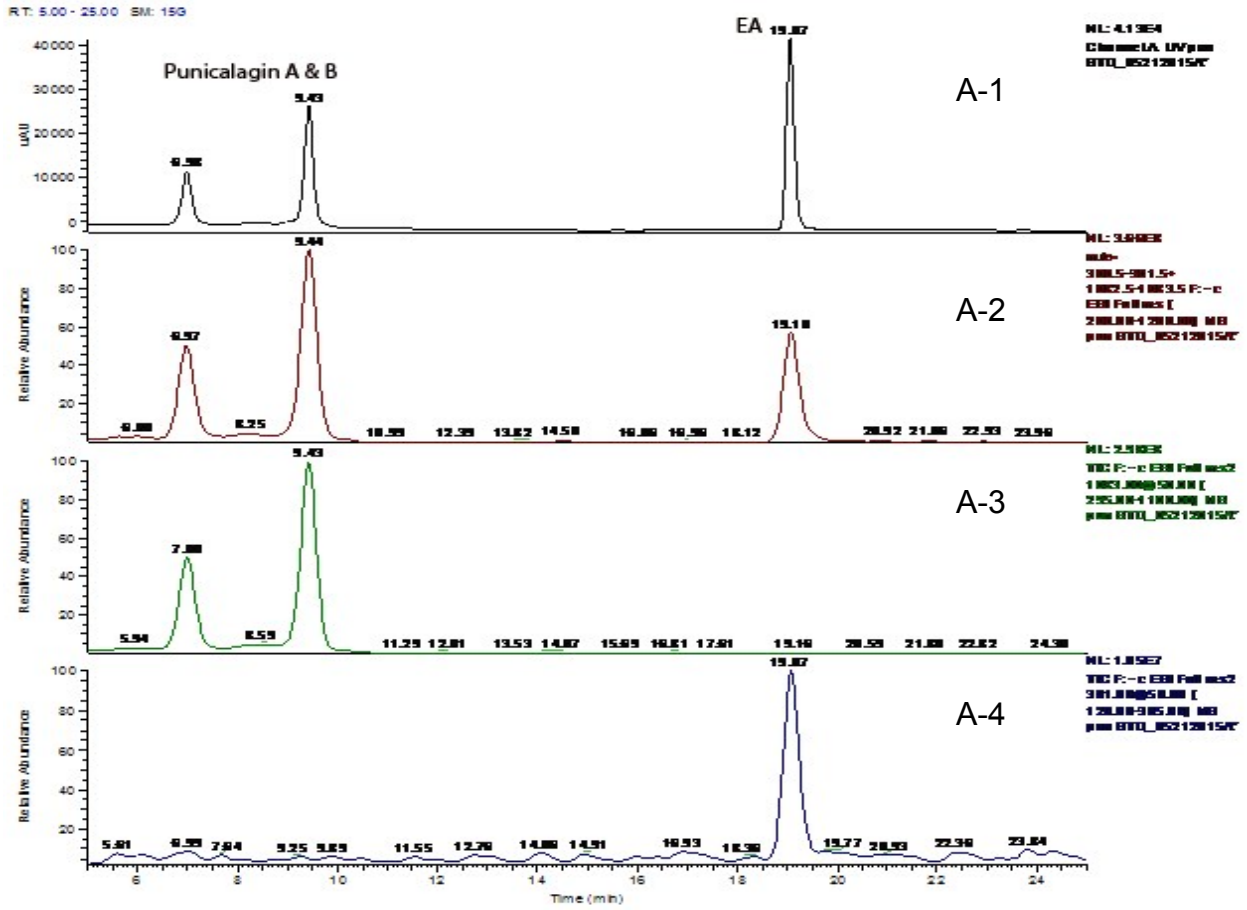
LC-MS/MS analysis of pomegranate extract

Five milligrams of pomegranate extract was dissolved in 5mL DMSO and further diluted 10 times in DMSO. The LC-MS/MS system consisted of a LCQ Advantage Finnigan instrument with ion-trap mass spectrometer equipped with an electrospray ionization (ESI) interface, and a Surveyor HPLC unit consisting of an autosampler/injector, quaternary pump, column heater, and diode array detector operated by Xcalibur software (Thermo Finnigan Corp). A Zorbax column (SB-C18 5 μ m 2.1x 150 mm column, Agilent) was used for the separation of analytes using a gradient elution condition by increasing the percentage of acetonitrile (with 1% acetic acid) in water (with 1% acetic acid) from 2% to 25% in 25 minutes at a flow rate of 0.2 mL/min. The MS conditions for the detection of ellagic acid and punicalagin A/B were as follows: ESI in negative mode; scan range 200 to 1200 amu; scan rate 1scan/second; cone voltage 17 eV. The identity of ellagic acid and punicalagin A/B was determined by matching the peak retention time and molecular ions [M-H]⁻ obtained by ESI/MS and tandem mass spectrometry (MS-MS) with the expected theoretical molecular weights and the data from literature (Nunez-Sanchez MA et al 2014). The deprotonated [M-H]⁻ ions were m/z 301 and 1083, and the fragment ions were m/z 257, 229 and 781, 601 for ellagic acid and punicalagin A/B, respectively.

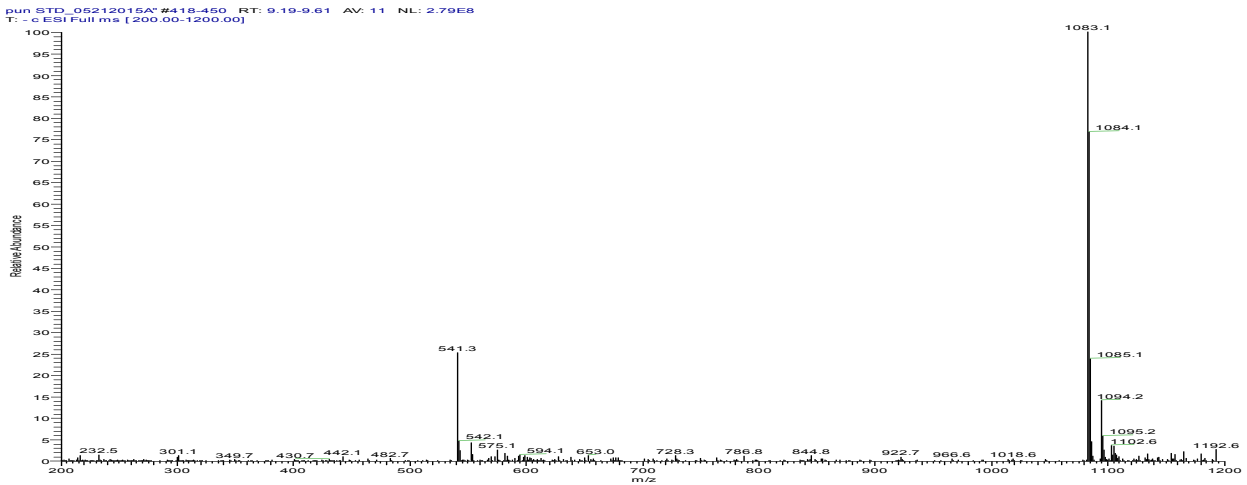
Nunez-Sanchez, M. A., Garcia-Villalba, R., Monedero-Saiz, T., Garcia-Talavera, N. V., Gomez-Sanchez, M. B., Sanchez-Alvarez, C., Garcia-Albert, A. M., Rodriguez-Gil, F. J., Ruiz-Marin, M. et al. (2014) Targeted metabolic profiling of pomegranate polyphenols and urolithins in plasma, urine and colon tissues from colorectal cancer patients. *Mol. Nutr. Food Res.* 58: 1199-1211.

Figure 1. LC-MS/MS analysis of standards (A) and pomegranate supplement (B).

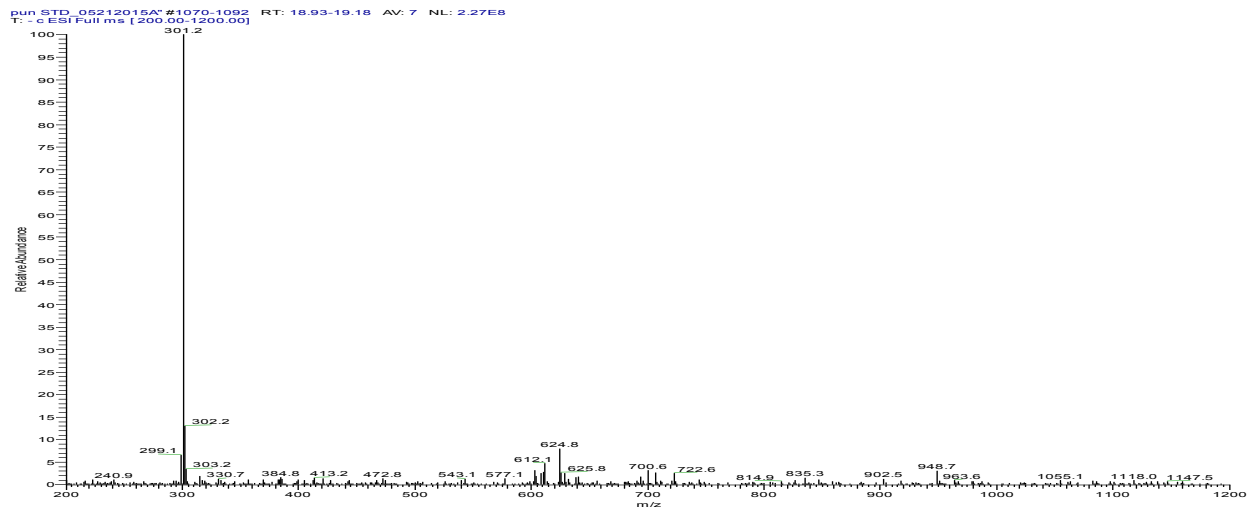
A. Standards



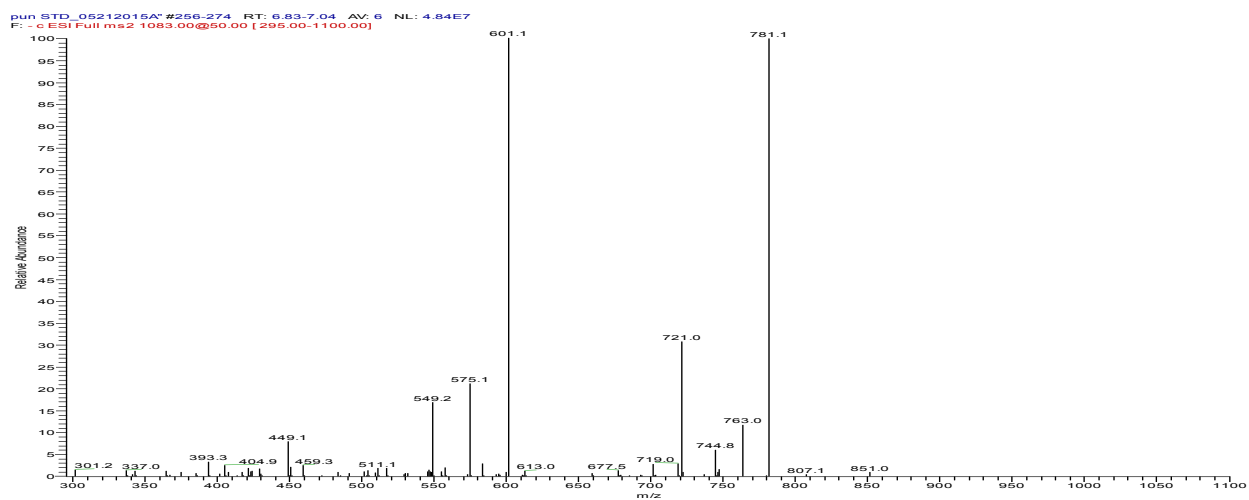
Punicalagin AB and EA standards: 1) UV @ 360 nm 2) Full Scan 200-1200 @ M-H
 M/Z=1083 & M/Z=301 3) MS/MS of 1083 4) MS/MS of 301



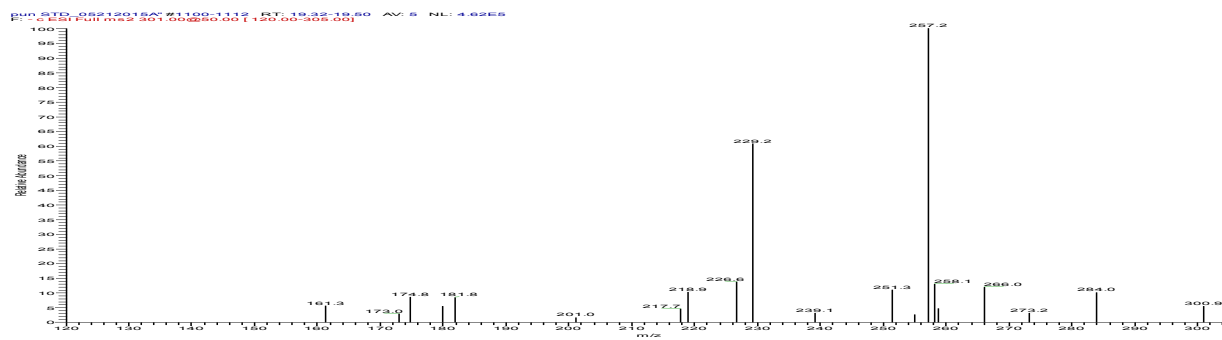
Spectrum of M/Z=1083



Spectrum of M/Z=301

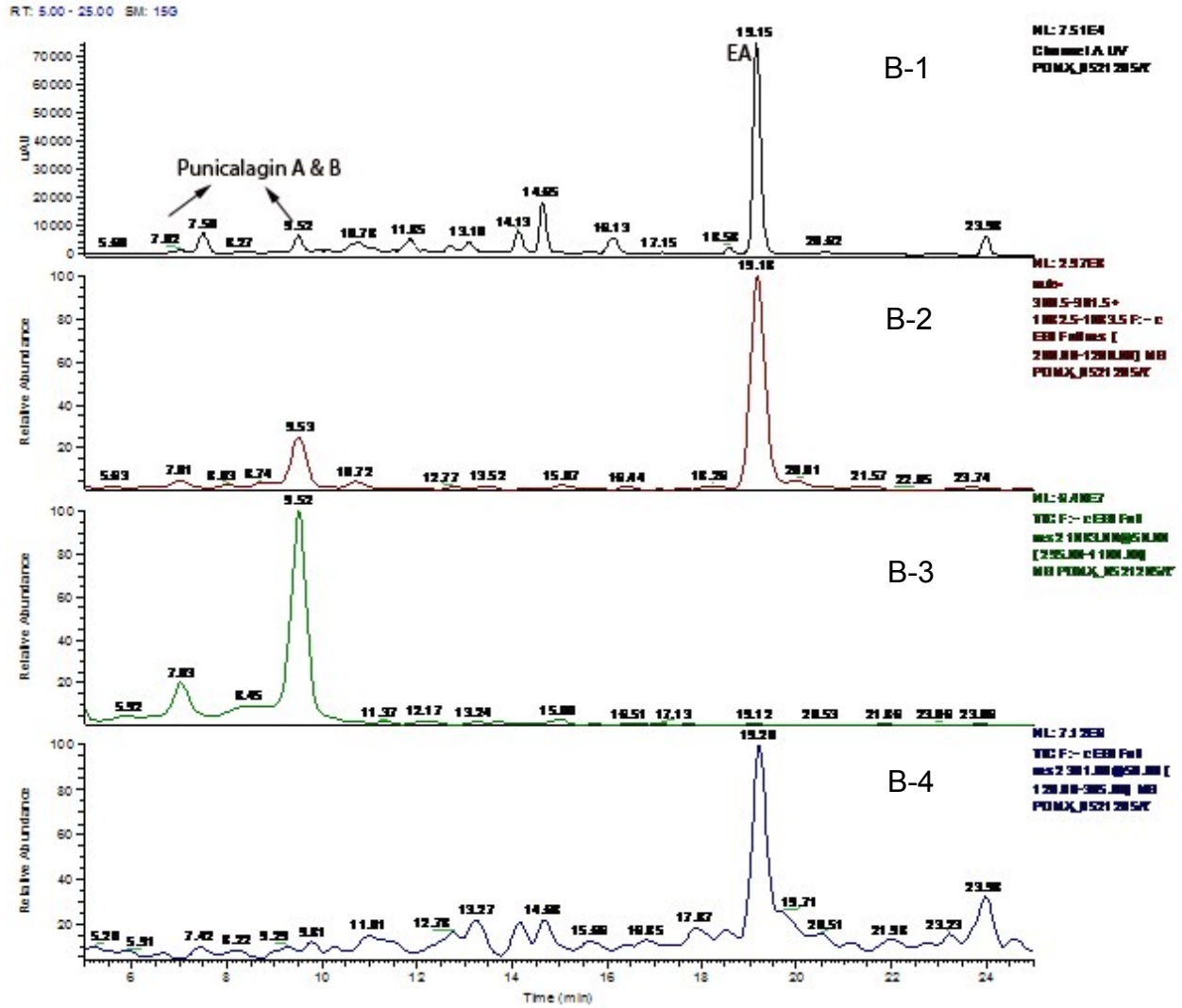


Spectrum of 1083 MS/MS



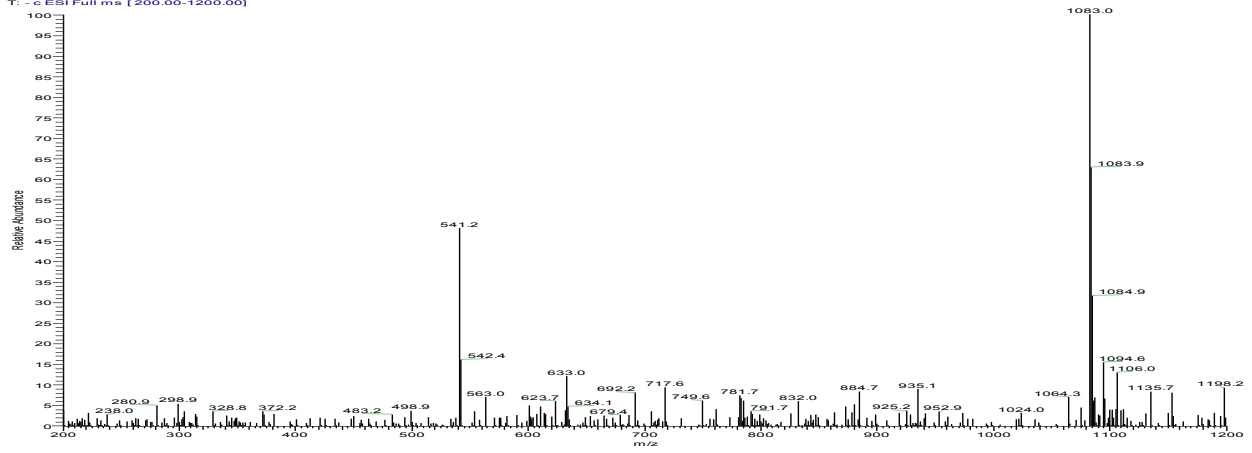
Spectrum of 301 MS/MS

B. Pomegranate extract



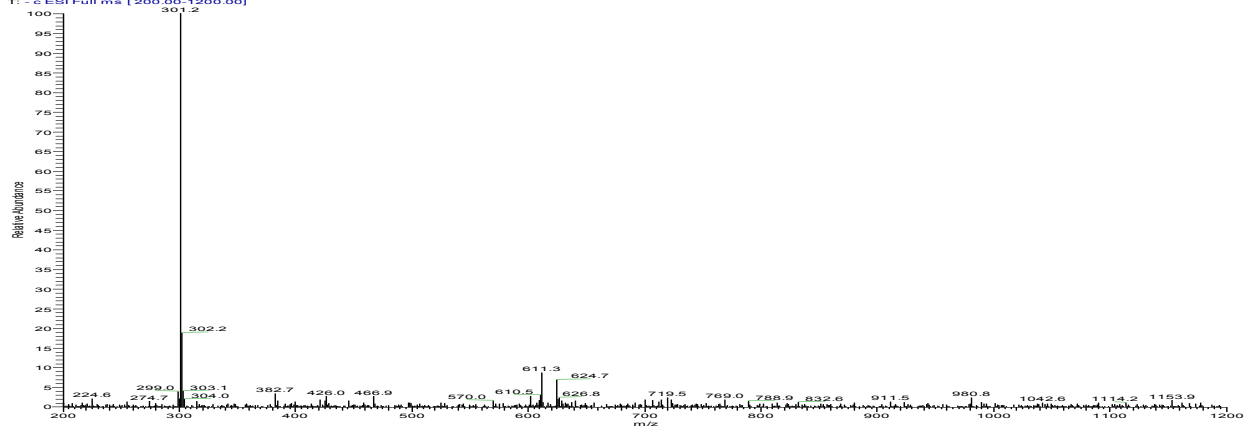
- 1) UV @ 360 nm
- 2) Full Scan 200-1200 @ M-H M/Z=1083 & M/Z=301
- 3) MS/MS of 1083
- 4) MS/MS of 301

POMX_0521205A#434.451 RT: 9.45-9.66 AV: 6 NL: 8.98E7
T: - c ESI Full ms [200.00-1200.00]



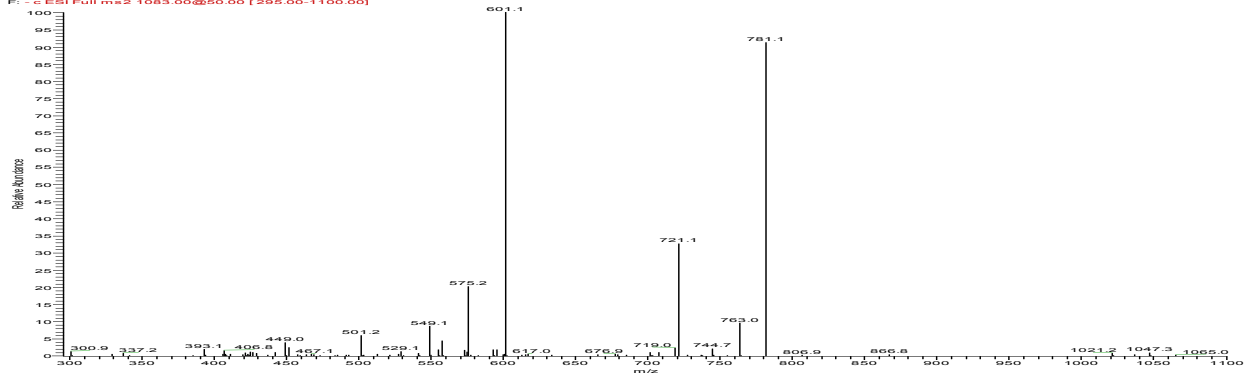
Spectrum of M/Z=1083

POMX_0521205A#1083-1111 RT: 19.01-19.39 AV: 10 NL: 2.46E8
T: - c ESI Full ms [200.00-1200.00]

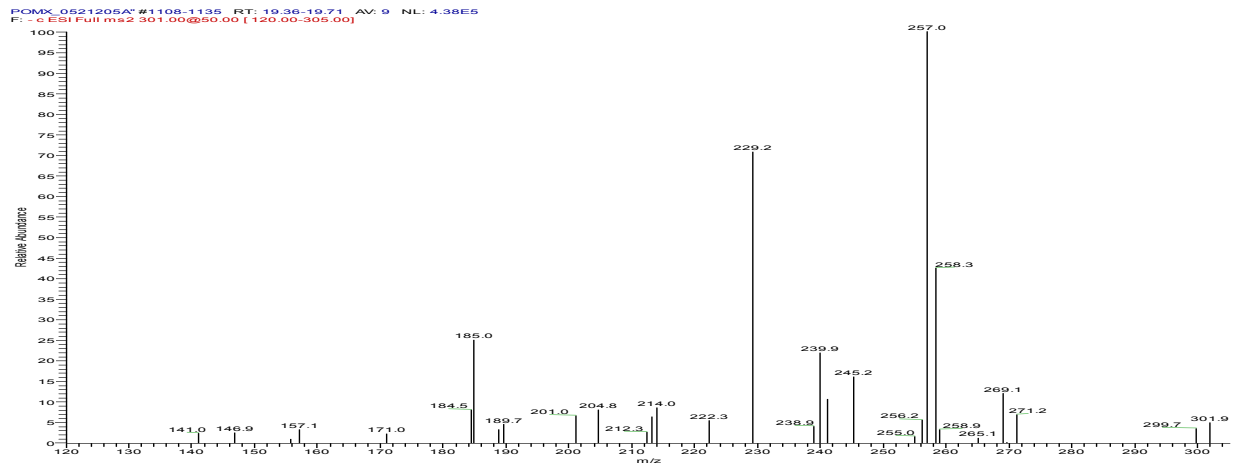


Spectrum of M/Z=301

POMX_0521205A#423.449 RT: 9.25-9.60 AV: 9 NL: 1.77E7
F: - c ESI Full ms x2 1083.00@50.00 [295.00-1100.00]

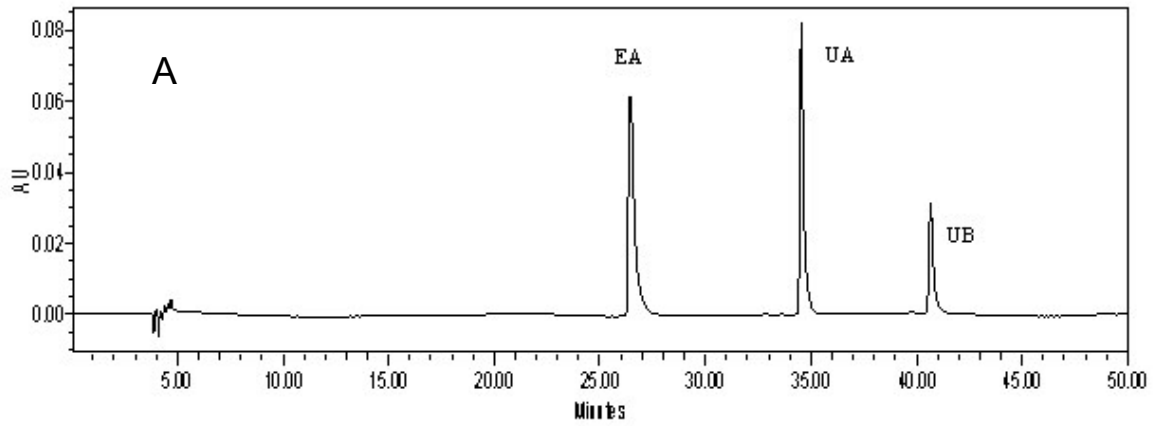


Spectrum of 1083 MS/MS

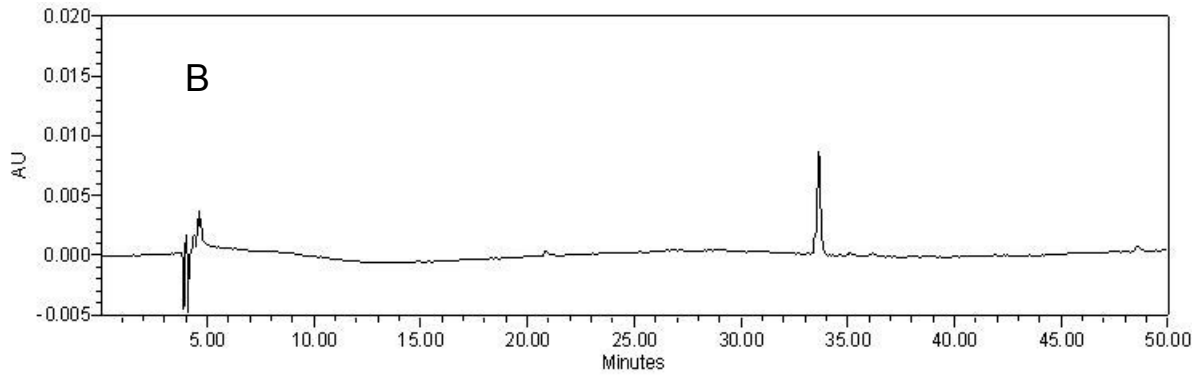


Spectrum of 301 MS/MS

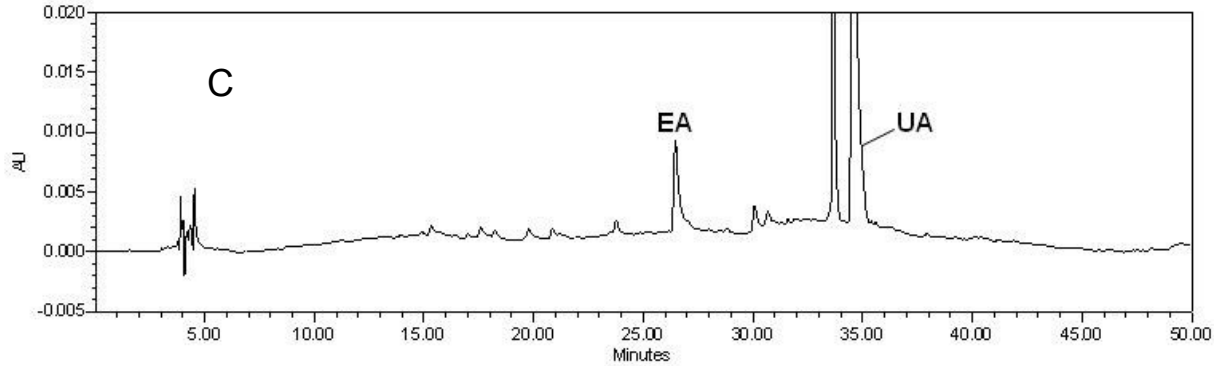
Figure 2. HPLC Chromatograms of standards (A): ellagic acid (EA), urolithin A (UA) and urolithin B (UB) and fecal samples (B-G) representing the different groups of UA formation.



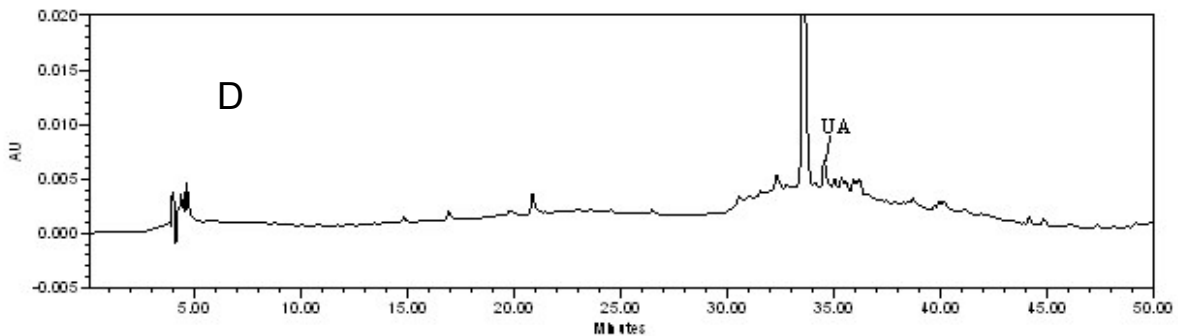
Example for Group 1: individuals with no baseline fecal UA presence (B) but induction of fecal UA formation by POM extract consumption after 28 days (C).
Baseline



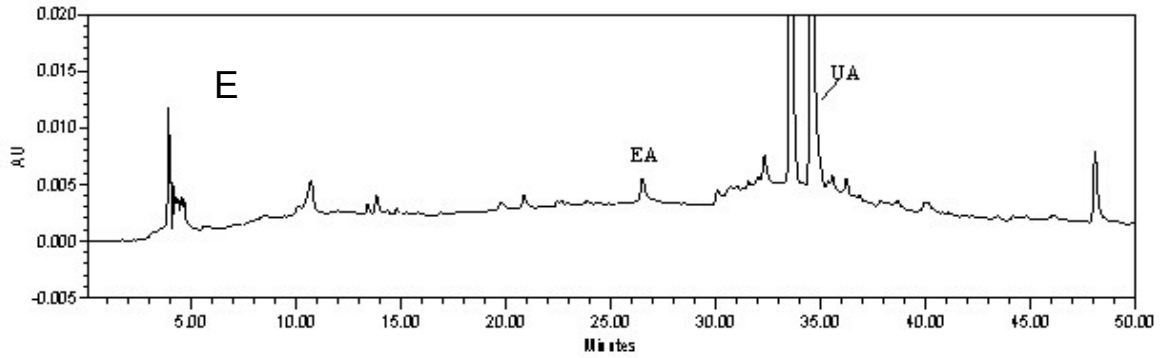
Day 28



Example for Group 2: baseline fecal UA formation (D) which was enhanced by POM extract consumption (E).
Baseline

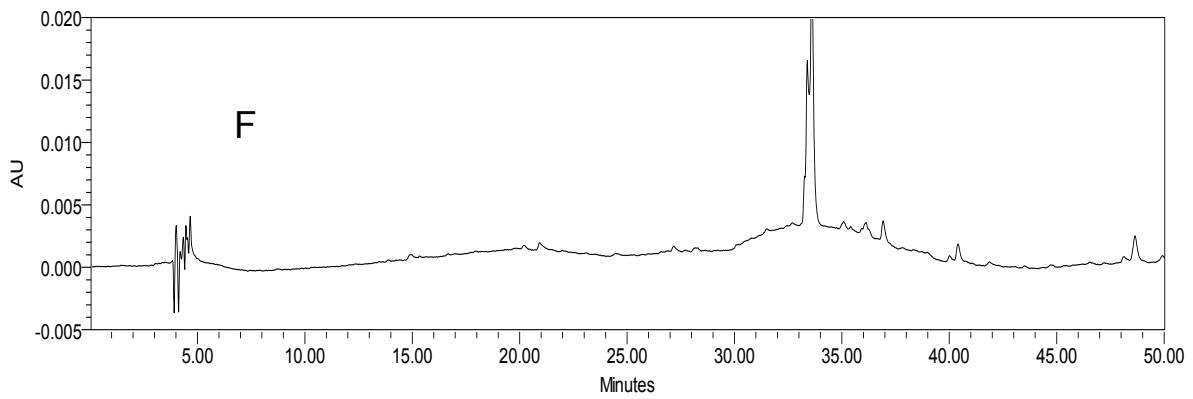


Day 28



Example for Group 3: no baseline fecal UA production (F), which was not inducible (G).

Baseline



Day 28

