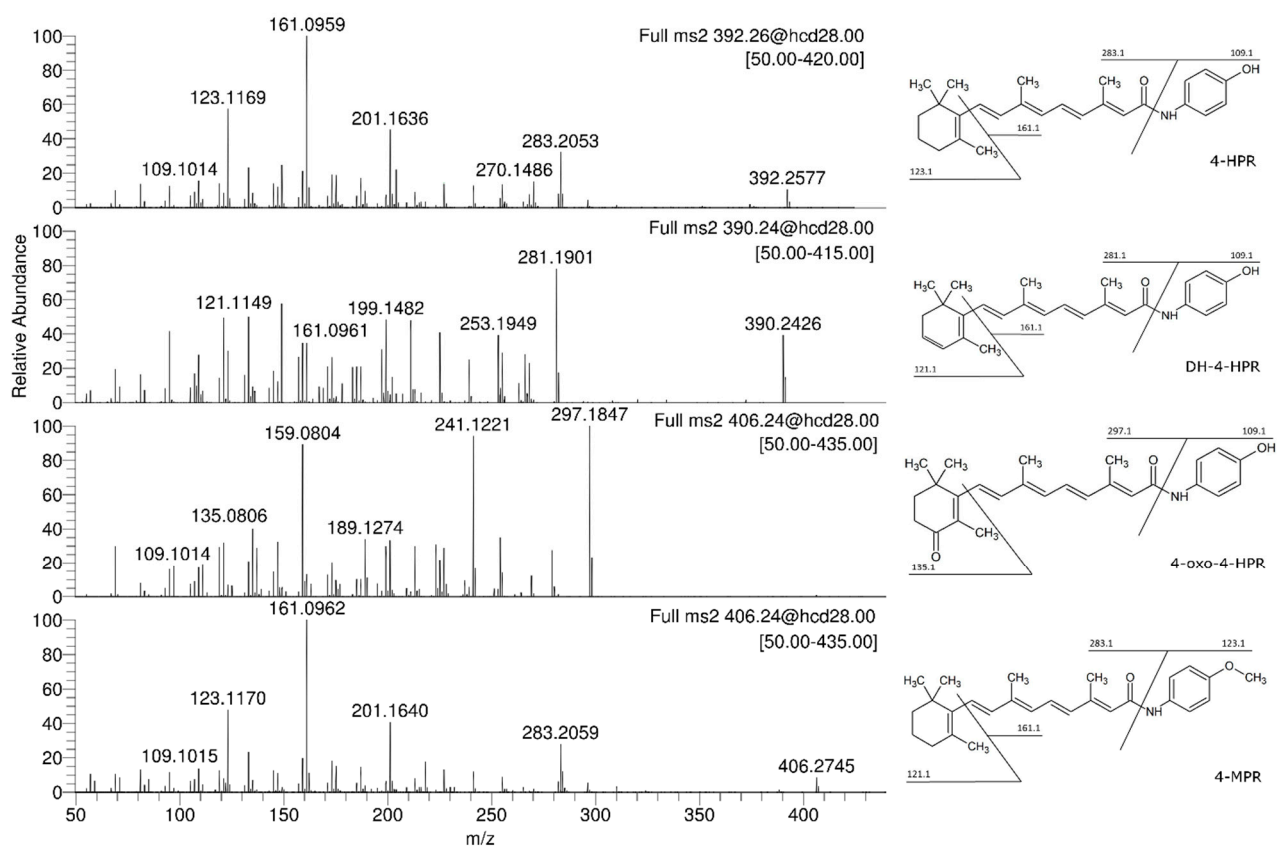


Supplementary Figure S1. High resolution ion chromatogram obtained for the m/z value (± 10 ppm) of 4-HPR and its main metabolites in a tumor sample.



Supplementary Figure S2. MS/MS spectra of 4-HPR and its identified metabolites, highlighting some of the observed ion fragments.

Supplementary Table S1: Comparison of the characteristics of the current method with others present in the literature

	Matrices analyzed	Analytes analyzed	Common features	Differences	LOQ in plasma	Improvements
F. Formelli et al., 1993 [12]	Plasma Breast tissue	4-HPR 4-MPR	<ul style="list-style-type: none"> - Handling procedures. - Chromatographic characteristics: reverse column, gradient features and run time below 10 min. 	<ul style="list-style-type: none"> - Extractive solvent: CH₃CN - HPLC-UV (λ= 340) - 3 points calibration plasma range: 5-2500 ng/mL. 	5 ng/ml	<ul style="list-style-type: none"> - Wider range of linearity applied reduces the need for dilutions. - The simultaneous measurement of the analyte and the metabolites allowed a more thorough analysis of the pharmacokinetic characteristics and limited the amount of matrix needed.
J. Vratilova et al., 2004 [13]	Tumour	4-HPR 4-MPR		<ul style="list-style-type: none"> - Extractive solvent: CH₃CN - HPLC-UV (λ= 340). - 6 points calibration tumor range: 0.5-20 µg/mL. 	0.5 µg/mL.	
J. I. Lee et al., 2008 [10]	Plasma	4-HPR 4-MPR		<ul style="list-style-type: none"> - Extractive solvent: CH₃CN - HPLC-APCI-MS/MS. MRM + - 8 points calibration plasma range: 0.5-100 ng/mL. - Target ions m/z 392.4/283.3 for 4-HPR and m/z 406.3/283.2 for 4-MPR. 	0.5 ng/ml	
H.E. Cho et al., 2017 [14]	Plasma	4-HPR 4-MPR 4-oxo-4-HPR		<ul style="list-style-type: none"> - Extractive solvent: CH₃CH₂OH - HPLC-ESI-QTRAP. MRM + - 8 points calibration plasma range: 0.2-50.0 ng/mL. - Target ions m/z 392.3/283.3 for 4-HPR, m/z 406.3/283.2 for 4-MPR and 406.3/297.2 for 4-oxo-4-HPR. 	0.2 ng/ml	
Present method	Plasma Tumour	4-HPR 4-MPR 4-oxo-4-HPR DH-4-HPR		<ul style="list-style-type: none"> - Extractive solvent: CH₃CN - HPLC-APCI-MS/MS. MRM + - 8 points calibration plasma range: 1-500 ng/mL. - 6 points calibration tumour range: 0.35-14.0 µg/g. - Target ions m/z 392.4/283.3 for 4-HPR, m/z 406.3/283.2 for 4-MPR and m/z 390.2/281.1 for DH-4-HPR. 	1.0 ng/ml	